

The Janus in the Soviet Camp

THAT science and politics are inextricably mixed in the Soviet Union is well known, especially to those who have regularly followed the pages of *Nature* in recent years. But in the past twelve months there has been evidence of an increased thaw in relations between the Soviet Union and the western world and it is sad that yet once again an event has occurred which is bound to set back the processes by which amiable interchanges between the Soviet Union and the West have increased.

The incident which has now cropped up concerns the treatment accorded Dr Evgeny Levich, son of Professor Benjamin Levich, and if all the details which have emerged from the Soviet Union are substantiated, it represents a complete reversal to all that was bad in the Stalinist era. Evgeny, whose trials began in March 1972 when he applied for permission to emigrate to Israel, has recently been whisked away to Tiksi within the Arctic circle to start a period of military service. But the unsavoury aspect of the entire affair is the way in which this was done without any apparent regard for Evgeny's medical condition. A year ago (see *Nature*, 238, 365; 1972) it was reported that he had a serious blood pressure condition but according to a recent telephone message from Professor Benjamin Levich his son's condition has now been diagnosed as being caused by ulcerous colitis and multiple diverticulosis of the bowels. As if this is not enough Evgeny is also reported to have a rectal tumour whose nature has yet to be described. This then was the condition of the young Levich when on May 16 he was apprehended on the street by what can only be described as a press gang which took him away leaving his wife helplessly behind.

But what can now be done? Professor Levich hopes that by means of a massive outcry from the scientific community outside the Soviet Union the authorities will be forced to return his son from Siberia to Moscow both to receive further medical treatment and to continue his work as an astrophysicist at the Chemical Physics Institute of the Academy of Sciences. But it must be asked whether this is a pious hope? Professor Levich himself feels that his position has been bettered within the Soviet Union following just such a campaign which was launched a year ago on his behalf when he was deprived of his university chair and of his position as head of the Institute of Electrochemistry in Moscow following his application—along with his sons—for permission to emigrate to Israel. It is true that recently Professor Levich's right to publish in the open literature has been restored but now there is news that he is faced with expulsion from the Soviet Academy of Sciences, on the grounds that he had engaged in activities harmful to the Soviet Union. These activities, apparently, were his refusal to protest against the efforts being made in Britain and elsewhere to publicize his case. These included a press conference called last summer by scientists who had met Professor Levich in Moscow and an appeal launched on his behalf by Professor D. B. Spalding of Imperial College. Professor Levich, however,

has had neither his chair nor his position as head of the Institute restored since the campaign began.

In spite of this superficial lack of success, has the campaign in fact prevented Professor Levich's plight worsening? Professor Levich himself is convinced that much worse would have befallen him if it were not for the interest his case has raised outside the Soviet Union. From the position of an onlooker, in which the rest of the scientific community necessarily finds itself, it is difficult to argue with this logic. It now seems that a much better test of the sensitivity of the Soviet authorities to outside pressure will be found in the case of Evgeny Levich. Here, at least, the aim is straightforward, namely to have him certified medically unfit for military service. The campaign will have been a success if Evgeny is indeed returned to Moscow for treatment and to resume his career.

But only time will tell if an all out campaign of publicity is the best way to help Evgeny Levich. What is unfortunate though is that it is highly unlikely that the many scientists working in the Soviet Union who are regular readers of this journal will have the opportunity of seeing this comment on the plight of one of their colleagues. It is only six months since *Nature* published proof that severe censorship of this journal takes place before it is circulated within the Soviet Union (*Nature*, 240, 372; 1972) and from other accounts it is an unknown occurrence for the journal to appear regularly week after week. If the Soviet authorities are really intent on making a success of the various formal and informal exchange arrangements which are now in existence between them and other countries they must show that they are willing to do away with some of the restrictions placed on the scientific community which have persisted since the days of Stalin.

100 Years Ago



Hail Storm

DURING the passage across us this afternoon of a thunder-storm moving at so great a distance above the earth that the thunder was very feeble and the lightning very faint, we had a great hail storm, which commenced with conical-shaped opaque stones of the size of peas, at 4^h 27^m (only lasting one minute), beginning again at 4^h 29^m with circular transparent stones having a small opaque nucleus (again only lasting one minute), followed at 4^h 33^m with flattened stones of the form of common acid drops, transparent, except a thin opaque envelope (which soon melted), and having externally in the centre a small rugged piece of ice.

E. J. Lowe

Highfield House Observ., Nottingham, June 3
From *Nature*, 8, 121, June 12, 1873.



OLD WORLD

New Hope of Beating Car Exhaust Emissions

FOR the past six months the National Engineering Laboratory and Shell have been driving a 1.8 litre Morris Marina around Britain whose carefully measured exhaust emissions almost meet the federal standards originally set in the United States for introduction in 1976.

After two and a half years work the two organizations have produced a device labelled Vapipe (heat pipe vaporizer) that, when fitted to a standard car, can reduce emission of carbon monoxide and oxides of nitrogen to the 1976 levels of 3.4 and 0.4 grams per mile respectively, although it has no effect on hydrocarbon emissions.

The technique is neat and simple, and the Department of Trade and Industry—NEL's boss—is already eyeing the export market.

The solution proposed aims to prevent the problem of auto emissions rather than cure it, and it is based around a system that Shell demonstrated last year (see *Nature*, **236**, 194; 1973). When the air/fuel ratio of a car is increased from its usual level of 15:1 to about 22:1 the flame temperature in the cylinder is reduced, and as a result, so is the production of CO and NO_x. But for a car to run at such a high ratio a perfect mixture of air and petrol is needed, or the car will backfire.

The principle of reducing emissions by this method has been established for some time, but the difficulty has been to find a method compact enough, and cheap enough to manufacture in bulk, that does not require large amounts of engine redesign.

One of Shell's early attempts worked quite well, but two tons of laboratory equipment were involved. But now, by the simple use of a heat pipe, the problem has been partially solved.

A closed tube is run from the exhaust pipe of the car to its carburettor. The pipe contains a fluid which is boiled by the heat from the exhaust gases and which then condenses at the carburettor end of the tube before flowing back to be reboiled. The heat at the carburettor end vaporizes the petrol into the air stream which has just passed through the carburettor and is about to enter the engine. Because it is vaporized the resulting petrol and air mixture is uniform and high air/fuel ratios can be achieved.

The system is not without its problems. The increase in air/fuel ratio

inevitably involves a loss of power, but this is balanced partially by more efficient combustion. Nonetheless, larger engines may be the order of the day. Against this has to be put a marked improvement in miles per gallon and a fall in octane requirements. One test car has even been run on lead-free petrol.

One distinct advantage of the system over other possible solutions is cost. No

one is prepared to quote an actual price, but the system is said to be considerably cheaper than the figure of £120 to £250 that has been put forward in the United States as the expected cost of cleaner cars.

The vaporizer still leaves the problem of hydrocarbons to solve. Engine redesign or catalysts seem the obvious answer, and it is possible that the new NEL and Shell system could be fitted

NUCLEAR POWER

Shell Buys In

THE Royal Dutch-Shell group has bought a \$200 million stake in nuclear power. In a deal completed in New York this week Shell has gone into a 50-50 partnership with the Gulf Oil Corporation in Gulf Energy and Environmental Systems Company (Gulf E and ES), the California based nuclear power division of Gulf.

Shell's money has bought it into the development of the High Temperature Reactor that Gulf has developed since 1967 at a cost of about \$250 million. The company already has orders for six HTRs on its books, even though its first full scale prototype—a 330 megawatt reactor at Fort St Vrain has not yet gone critical. The orders—totalling 5,400 MW—should provide Gulf and Shell with the start of a commercial return for their money by the beginning of the 1980s. The nuclear fuel side of Gulf's business should also provide a return by the end of this decade.

The move by Shell reflects two things. First, its growing awareness that the future of oil is limited, even if there are enormous profits still to be made, and second, its faith in the future of nuclear power. Although Gulf has only been in the nuclear business for six years (it bought out General Dynamic's nuclear division in 1967), its work on HTRs has by-passed the light water reactors, and the bulk of Gulf's interest is concentrated in HTRs, ancillary equipment, and the development of fusion.

Although Shell's initial investment is \$200 million dollars, if the HTR sells as well as the two oil giants expect, Shell's investment could increase significantly. Gulf already holds option orders on two more 1,160 MW units, and Gulf E and ES has already concluded agreements in West Germany and France whereby their HTR designs will be marketed,

built and fuelled in Europe by French and German companies.

British Nuclear Design and Construction, one of the two nuclear consortia in Britain that are to be merged into one company under Sir Arnold Weinstock's GEC, have had close links with Gulf for some time. Twelve BNDC engineers visited Gulf's stations last year, and exchanges of information on pressure vessels and gas technology—which BNDC knows a lot about with its experience of building AGRs—have taken place. Taylor-Woodrow, a member of the BNDC consortia, already has a technical agreement with Gulf on pressure vessels.

Shell's choice of partner is partly one of necessity and partly one of skill. Neither Westinghouse nor General Electric, the two United States nuclear power giants, would be likely to want a new partner whereas Gulf, itself an oil company coming to terms with the future and calling itself an "energy" company, welcomed the additional investment and the use of Shell's worldwide contacts. The other advantage of joining Gulf is that HTRs have not been challenged on environmental and safety grounds to the same extent as the light water reactors which have been Westinghouse and GE's moneymakers.

Shell decided against starting off its own nuclear business, a spokesman said this week, because "extensive studies showed that we could not expect at this stage to develop our own nuclear technology on a reasonable time scale and on a competitive basis".

Apart from the HTR, Shell's stake in Gulf also provides it with an interest in the development of the fast breeder reactor, in the use of HTR as a source of process heat for industry, in light water fuel fabrication, and in direct cycle nuclear plants.

The agreement does not, however, include uranium mining and milling or enrichment services.

to existing cars with a catalyst added on to clean up the hydrocarbons, while new cars could be designed to produce fewer hydrocarbons with the new vaporizer included as part of the design.

If Vapipe does prove to be a commercial proposition, and if a suitably cheap catalyst to cope with the hydrocarbon emissions can be developed, then there must be a real possibility that the United States standards for 1975—which are easier to meet than the 1976 standards but which were still put back a year by Mr William D. Ruckelshaus, administrator of the Environmental Protection Agency (see *Nature*, **242**, 491; 1973)—will be introduced, in spite of the fervent opposition coming from Detroit.

CONSERVATION

Sargassum Saga

THE first attempts to clear the Japanese seaweed *Sargassum muticum* from the shores of the Isle of Wight have been successful. But now that the adult plants have been cleared, a two or three year campaign will have to be run to clear the young plants that are already springing up round the coast.

The seaweed, which is not indigenous to Britain, was first discovered in February by members of Portsmouth Polytechnic (see *Nature*, **243**, 231; 1973). Experience in British Columbia where the seaweed arrived in the 1940s and spread rapidly down the coast, lead to fears that it might also grow uncontrollably in British waters, forcing out other marine life, interfering with shipping, and defacing beaches.

To combat this, a group of marine scientists decided to attempt to eradicate the plant before it took a firm hold, and two to three tons of the seaweed were removed during the low tides at the end of May and the beginning of June.

The problem now is how to finance the long campaign to remove the young plants that are springing up. Unfortunately the weed could not be removed before it entered its fertile period and spores from the *Sargassum* have escaped and young plants are already growing again at Bembridge, where the seaweed was originally discovered.

Sargassum grows thick and fast, at the rate of two centimetres a day. Parts of the Bembridge ledges that were clear last Thursday had young plants five centimetres long established by Sunday.

Dr Gareth Jones of Portsmouth Polytechnic, who has organized the eradication attempt, said this week that now all the adult plants have been removed from Bembridge, St Helens and Shanklin the need is to train about a dozen people to recognize the leafy young plants so that they can be cleared at fortnightly

intervals when the tides are right in order to prevent the seaweed taking root again. At present of those who live locally and helped with the clearance only Dr Jones, and Dr Bill Farnham who discovered the *Sargassum*, are able to recognize the young plants which look rather different to the bushy adult weed which has been known to grow up to 10 metres long outside Britain.

The other problem is finance. With the help of teams from the University of Liverpool, North London Polytechnic and local sixth formers, the adult plants have been cleared at a cost of about £200. But Portsmouth Polytechnic can no longer afford to finance the eradication and more money is urgently needed. The most likely sources are the Nature Conservancy and the local councils on the Isle of Wight, but unless something like £500 a year can be found it will be difficult to ensure that the plant does not reestablish itself.

Dr Jones said this week that certain areas of the island—notably Totland Bay and Gunard Ledges—still need searching to ensure that adult plants have not grown up there.

Meanwhile the Natural Environment Research Council has given the Polytechnic a small grant with which to try and find out where the weed came from. Studies of the effect of *Sargassum* on other marine life—notably eel grass—will also be carried out.

The presence of *Sargassum* has highlighted a gap in government responsibility. Because it grows between high and low water marks, local government is not responsible for clearance, and central government can only be involved when the seaweed is proved to be a pest, by which time eradication would be impossible. Without the action of private individuals, no one would stop the weed establishing itself.

Short Notes

Windlesham succeeds Jellicoe

LORD WINDLESHAM has been appointed Leader of the House of Lords and Lord Privy Seal following Lord Jellicoe's resignation a fortnight ago. Lord Windlesham, a former director of Rediffusion Television and managing director of Grampian Television has been

Minister of State at the Home Office since 1970. In succeeding to the job of Lord Privy Seal, Lord Windlesham will almost certainly take responsibility for the Civil Service Department, and may take charge of the changes that were introduced in last July's White Paper on Government Research and Development which Lord Jellicoe formulated and saw through Parliament, although the task of being the Government's spokesman on science does not necessarily fall to the Lord Privy Seal.

Stewart to chair ABRC

PROFESSOR F. H. Stewart, Chairman of the Natural Environment Research Council, is to succeed Sir Frederick Dainton as Chairman of the Advisory Board for the Research Councils. Sir Frederick was appointed Chairman of the University Grants Committee earlier this year.

Professor Stewart, who will take up his appointment in October, has been Chairman of NERC since 1971. He is also Regius Professor of Geology at the University of Edinburgh. In his capacity as Chairman of NERC, Professor Stewart already holds a seat on the advisory board, and he was a member of the ABRC's predecessor, the Council for Scientific Policy, from 1967.

Honours for Scientists

J. A. B. GRAY, Secretary of the Medical Research Council was one of five scientists knighted last week when the Birthday Honours List was announced. Dr Gray, who has been the MRC's secretary since 1968, was made a knight bachelor, as was Professor Raymond Firth, Professor of Anthropology at the University of London, Dr Percy Kent, formerly Chief Geologist and Exploration Manager to British Petroleum, Dr James Menter, Director of Research and Development at Tube Investments, Vice-President of the Royal Society and former President of the Institute of Physics, and Professor Eric Scowen, chairman of the Committee on Safety of Medicines. Earlier last week Sir Eric Ashby, Master of Clare College, Cambridge, since 1959, and former Chairman of the Royal Commission on Environmental Pollution, was made a life peer.

Professor Herbert Dingle : An Apology

IN *Nature* of January 12, 1973, we published a review by Professor John Ziman of Professor H. Dingle's book *Science at the Crossroads*. Though most of the review consisted of a discussion of ideas put forward in Professor Dingle's book, at one point in the review the book was described as "dishonest". We accept that there was no basis for attacking Professor Dingle's personal integrity and accordingly both *Nature* and Professor Ziman offer their sincere apologies to Professor Dingle.

NEW WORLD

Another Pesticide in Trouble

by our Washington Correspondent

THE series of battles that have been taking place in the United States over the safety of pesticides bears a strong resemblance to the interminable run of James Bond films: the same cast of characters appears in the same situations, and each successive production is as absorbing as its predecessor. Last year, the big attraction was the trial of DDT; this year, the trial of aldrin and dieldrin will soon begin its opening run in Washington. Unlike the James Bond films, however, there is more at stake than mere entertainment value, and the outcome is not always as predictable.

A trailer for the aldrin and dieldrin epic was given in Washington last week, when lawyers for the parties in dispute over the risks and benefits of the pesticides submitted their prehearing briefs to a hearing examiner. A public hearing will open soon—the date has not yet been decided—but the pretrial briefs outline the arguments which will be used by the Environmental Protection Agency and the Environmental Defense Fund, who want to get the pesticides off the market, and the Department of Agriculture and the Shell Chemical Company who want to continue using them. The public hearing will be the latest—perhaps the final—stage in a prolonged dispute over the pesticides.

The story so far is that in June last year, William D. Ruckelshaus, then Administrator of the Environmental Protection Agency, cancelled all registered uses of the pesticides except for mothproofing, termite control and some nursery uses. Shell, the sole manufacturer, objected to the cancellation notices and asked for a public hearing. The issues which will be debated are remarkably similar to those which came up in the prolonged dispute over DDT.

Aldrin is an organochlorine pesticide which is degraded fairly rapidly to the epoxide dieldrin, which is extremely long lived. It is used as a pesticide in both forms, and they will be considered together in the forthcoming public hearing. The chief use of the pesticide is to control pests in the soil to prevent damage to corn, and other uses in dispute are the treatment of seeds and the control of soil pests in the cultivation of citrus fruit in Florida. According to the brief from Shell, about 8 million acres of corn fields are treated with aldrin each year at a cost of about \$1.50 per acre, and it is also used in about 30,000 acres of citrus. In all,

some 10 million pounds of the pesticide is used each year, which is, less than the 20 million used in 1966.

In short, the problems with aldrin stem from the extreme persistence of its breakdown product, dieldrin, which leads to the familiar biomagnification in the food chain, alleged damage to wildlife and, what will undoubtedly be the crucial point in the hearings, its chronic toxicity to man. As with DDT, the most hotly debated topic is likely to be the possible cancer hazard.

Lawyers for Shell will argue at the hearings that the pesticide only poses a problem because of incorrect or careless usage. Their prehearing brief points out that when used to control soil insects in corn growing areas and in Florida, the pesticide is buried three inches below the surface, which should be sufficient to prevent it from being transported by agricultural runoff. They will also argue that there are no alternatives, and that there is no way of telling which fields are likely to be attacked by soil pests, hence farmers must use it before an outbreak as insurance.

But lawyers from the EPA will beg to differ. Their brief promises to reveal evidence of heavy concentrations in water, and in many aquatic species and in some birds. As further evidence of transportation, they will show that dieldrin is found in Antarctic penguins and whales. More important, an EPA study in 1971 turned up evidence of dieldrin residues in adipose body tissues of 99.75 per cent of the US population, and another study found residues in 83 per cent of the composites of meat, fish and poultry and 80 per cent of all milk samples consumed in the US.

Those figures will be the starting point for the EPA's arguments about the possible chronic toxicity of dieldrin to man. The evidence for possible carcinogenicity derives chiefly from long term feeding studies conducted on mice and one study (which the Shell brief curiously neglects to mention) conducted by the Food and Drug Administration on rats. Most of the key mice experiments were in fact conducted in Shell's own laboratories, but Shell and its opponents differ in the interpretation of the results, and the public hearing seems destined to develop into another debate about the applicability of animal studies to man. In many respects, the arguments are similar to those which continually circulate about the safety of food additives.

The EPA and EDF lawyers take the

position that it is usually impossible to prove with absolute certainty that a chemical is carcinogenic to man, but if it is found to be carcinogenic to test animals then it must be regarded as a danger to man. As the EDF brief points out, "only a human catastrophe can prove conclusively that a chemical is carcinogenic to man". In the case of dieldrin, several mouse feeding studies have turned up evidence of liver tumours, while the FDA rat study suggested an increase in the incidence of tumours at various sites.

Shell's lawyers maintain, however, that the evidence of liver tumours in mice "says volumes about the mouse, (but) is not evidence of human tumorigenicity . . . the mouse liver tumours here are naturally occurring. Dieldrin merely increased their incidence". Shell backs up its case with data derived from a study of workers in an aldrin/dieldrin production plant, exposed to high concentrations of the pesticide. The study has turned up no evidence of liver tumours, or other signs of disturbance of liver function. But the EPA document points out that this study was carried out on "presumably healthy adults and the duration of their exposure is insufficient for an assessment of important chronic effects".

In many respects, the forthcoming hearings will boil down to a debate over the important philosophical distinction between socially accepted level of risk, and safe level for man. It should be remembered that in the case of DDT, the hearing examiner determined that use of that pesticide constituted a socially acceptable level of risk, and consequently recommended that DDT should continue to be used. Mr Ruckelshaus decided, however, that there was no safe level for man, and banned DDT. Whether the arguments over aldrin/dieldrin will be as finely balanced remains to be seen.

NIH

Unknown Quantity

by our Washington Correspondent

For the past few months, several scientists at the National Institutes of Health have been up in arms about the health policies of the Administration. Their disaffection first set in last December when Dr Robert Q. Marston was informed by the White House that he was being fired as the NIH Director, and it

turned from bad to worse in January when the Administration's budget was unveiled to reveal savage cutbacks in a variety of biomedical research programmes. More recently, considerable alarm has been generated at NIH by a memorandum from the Office of Management and Budget attacking the peer review system by which grant proposals are evaluated for scientific merit (see *Nature*, 243, 257; 1973).

It is thus a troubled agency that Dr Robert S. Stone inherited last week when he was officially confirmed as the new NIH Director. A pathologist who has been Dean of the School of Medicine of the University of New Mexico for the past five years, Dr Stone's appointment has not caused much of a stir at NIH. He is very much an unknown quantity and the general attitude there seems to be to wait and see. But some scientists polled last week said that they are at least happy that he has had some research experience (in tumour virology, etiology of lung diseases and development of a regional plan for meson radiotherapy of cancer), unlike a few of the candidates who were rumoured to have been in the running for the job.

One of the chief reasons for discontent at the firing of Dr Marston last year was that it is widely suspected that he was removed by the White House essentially because he voiced opposition to some Administration policies (no official reasons have ever been given) and Dr Stone's public statements and positions will be carefully watched to see whether he will toe the Administration line. At his first meeting with the press last week Dr Stone gave few things away.

He maintained several times that he has been given no instructions about policy from the White House or from the Department of Health, Education and Welfare, and said that he expects to voice his own opinions vigorously in the formulation of policy. As for a specific issue which has been troubling several people, namely that the National Cancer Institute and the National Heart and Lung Institute between them soaked up all the increase in the NIH budget while other institutes suffered cuts, Dr Stone merely said that the balance of funds between institutes and between programmes needs to be continually under review. He added, however, that "it is less important where the money is, as long as a substantial amount is going into fundamental research".

Turning to the question of training grants, Dr Stone did not say outright whether or not he agrees with the Administration's decision to phase them out entirely. The NIH is on record, however, as vigorously defending training grants last year in the preparation of the budget document, but it lost the battle to the Office of Management and



Dr Robert S. Stone, New Director of the National Institutes of Health.

Budget. Dr Stone acknowledged that training grants have been very important "in building up a cadre of scientists", and he added that if the question is reopened, he will "bring my viewpoint on what training grants have accomplished", which seems to come close to suggesting that he will fight for them to be reinstated.

Finally, on the question of peer review, Dr Stone praised the accomplishments of the system and said that he is "clearly in support of peer review—I don't know what we can substitute for it". Specifically in regard to the OMB memorandum, he said "my bias is that scientists around the country have been reviewing each other's work with a high level of objectivity. I would be very careful about substituting anything for peer review".

Dr Stone brings to the National Institutes of Health considerable experience as a scientist-administrator. Born in 1922, he received his BA degree from Brooklyn College, New York, in 1942 and his MD from the State University of New York College of Medicine in 1950. He moved to UCLA School of Medicine in 1952 and in 1963 was appointed Chairman of the Department of Pathology at the University of New Mexico. He became Dean of the School of Medicine there in 1968, managed to attract good staff, and built it up during a difficult financial period.

Short Notes

Lunar Radio Astronomy

A SATELLITE designed to study low frequency radio signals from galactic and extra-galactic radio sources is set for launch at the end of this week. Called Radio Astronomy Explorer-B (RAE-B), it will be placed in lunar orbit and will

study cosmic radio noise in the 0.02–13 MHz region. The satellite is a follow up to Explorer-38 which studied low frequency radio noise for four years after it was launched into Earth orbit in 1968. A lunar orbit was chosen for RAE-B because interference from radio noise from Earth saturated the receivers on Explorer-38 for between 25 and 40 per cent of its viewing time, and also solar activity after it was launched was greater than expected. The idea is that the Moon will shield the satellite from Earth radio noise, but the lunar orbit also has the advantage that lunar occultation can be used to pinpoint radio sources. The satellite, which cost \$8.6 million, is due to be launched from the Kennedy Space Center on June 9.

TA in Commerce

THE Department of Commerce has set up a new Office of Technology Assessment and Forecast, chiefly to analyse and disseminate information contained in the files of the US Patent Office. A preliminary report published last week suggests that the premise underlying the new office's work is that changing trends in the patents filed at the Patent Office can be an accurate indicator of technological activity throughout the world. The office will thus seek to identify those areas of technology which have unusually rapid growth and also those areas in which a high proportion of patent activity originates outside the US. The idea will be to publish reports of patent activity in such fields. According to Dr Betsy Anker-Johnson, the new Assistant Secretary of Commerce for Science and Technology, other types of data, such as census reports and export and import figures, may be added to the system later. The office is being directed by Mr Alfred C. Marmor.

NSF to the Rescue

The National Science Foundation has granted a stay of execution to the Space Radiation Effects Laboratory in Newport News, Virginia. A 600 MeV proton accelerator operated for NASA by William and Mary College's Virginia Associated Research Center, SREL, was due to be closed down on June 30 because of lack of money. NSF is to put up \$260,000 and William and Mary will put up another \$145,000 to keep the machine running for another year, because the NSF considers it to be "an important intermediate energy facility". The accelerator was built in the mid 1960s at a cost of \$15 million and it is used to simulate particle interactions in the Earth's outer atmosphere. The NSF grant only extends for a year, however, and in view of the failure of other accelerators to struggle on without federal grants, SREL's days are probably numbered.

NEWS AND VIEWS

Machine Nous

AFTER two months of "such limited studies as he was able to make", Sir James Lighthill (*Artificial Intelligence*, SRC, April 1973) has rejected the claim of artificial intelligence to special consideration by the Science Research Council on grounds which differ remarkably little from Lady Lovelace's objection to Babbage's analytical machine in 1842 (Turing, *Mind*, **59**, 433; 1950). Apparently it is as true today as it was more than a century ago that what you get out of a machine depends on what you put into it: as Lady Lovelace has it, "The Analytical Engine has no pretensions to *originate* anything. It can do *whatever we know how to order it to perform*"; or according to Lighthill, modern machines can successfully "... perform in highly specialized problem domains, when the programming takes very full account of human experience and human intelligence within the relevant domain ...".

He goes on, however, to describe as the "coveted long-term goal of AI" the development of "a general-purpose program seeking to mimic the problem-solving aspects of human CNS activity". It is on the grounds of what he feels to be inadequate progress towards this goal that Lighthill concludes that further support for basic research on artificial intelligence as such—that is, as distinct from advanced automation or theoretical neurobiology—is unjustified. This immediately exposes him to the criticism that mimicking human intelligence is not the goal of artificial intelligence research, which is concerned with intelligence *per se*, and not necessarily as implemented in the human brain.

Be that as it may, it is entirely understandable that an outsider should come away with the impression that mimicry is the ultimate aim, when according to many insiders the crowning achievement in the field so far is Winograd's program for conversing with a machine in natural language (see *Nature*, **242**, 372; 1973; and Winograd, *Understanding Natural Language*, Edinburgh University Press, 1972). The excitement engendered by this development is acknowledged by Lighthill in a post-script considering separately the claim made for the program as vindicating artificial intelligence research.

The success of Winograd's program in dealing with the complexities inherent in the ordinary use of English depends partly on its having access to information on the universe of discourse: its behaviour is guided by the context within which it is working, as would be the case with human behaviour, and not simply by the input and its own intrinsic logic. In spite of this gift of human nous, Lighthill points out, it took a large computer a long time to make simple conversation about coloured blocks on a table top; and Lady Lovelace's objection still stands.

Thus Lighthill concludes that the results of work on artificial intelligence remain disappointing, and adds, more contentiously, that its goals are as remote as ever: a guess which is presumably based on Lighthill's general impression of the quality of work in the field. Professor N. S. Sutherland, in a cogent if somewhat waspish critique of Lighthill, raises the possibility that his impres-

sion is a function of the singers rather than the song. Sutherland bluntly states, "There is little first class work on basic AI in progress in Britain at the moment"; the leading workers in the field are mostly in the United States at Stanford or the Massachusetts Institute of Technology, and according to Professor D. M. Michie, who also submitted his comments, Lighthill did not actually speak to them.

Sutherland argues vehemently the importance of studying intelligence without the constraints of its mechanism in living brains (which, he points out, can generate some extremely unintelligent behaviour). In concrete terms, artificial intelligence research has produced some extremely sophisticated programming techniques which Sutherland believes have general application. Lighthill does not dispute the contribution of AI research to the understanding of natural language; on the same principle, Sutherland emphasizes the insight into the processes of thought and perception gained by the attempt to formalize them.

As far as Sutherland is concerned, the source of most that is good is the group at MIT headed by Marvin Minsky and Seymour Pappert, within which the ideas and techniques on which Winograd's program is based were developed. This view is reflected strongly in his suggestions for the deployment of research funds. These include importing young workers from the United States, the provision of computers which can accept US software, and sending young Britons for training to MIT. One role which Sutherland envisages for basic AI centres is in the training of young workers to supply the other related research areas of advanced automation and theoretical investigation of the central nervous system.

It is fair to say that progress in artificial intelligence has been unamazing; but in the twenty or so years that have elapsed since Turing considered the question of whether machines could think, and concluded with the suggestion that a machine might be built to play chess, or to understand English, machines have been developed that play chess, albeit only at the level of the talented amateur; and now they can also speak English, if only with the voice of the programmer. Progress in the next twenty years will doubtless depend on financial support for workers of Turing's calibre.

Probing in the Rain

THE increasing demand for telecommunications capacity in Earth-satellite and terrestrial networks requires the extension of present services to carrier frequencies above 10 GHz. Not only do new channels then become available but practical bandwidths are compatible with the needs of high-speed transmission of digital information.

An important problem in the development of the new services is the attenuation of the signals with absorption and scattering of energy which occurs due to precipitation in the transmission path. Rain, for instance, is

highly variable: it may fall at a low rate as in drizzle or at a high rate in thunderstorms, be widespread over an area or of limited extent in showers, and its intensity and duration differ considerably from place to place and from one occasion to another. This variability makes it difficult to obtain good estimates of the reliabilities likely to be achieved by particular millimetre-wave communication systems. Transmissions must be maintained for a high proportion of the time, and the systems must remain operational through all but the extreme conditions of precipitation which may occur very rarely.

Although measurements of rainfall have been accumulated for many years now, they often refer only to the total amounts falling at different places during intervals which may be as long as a day and are rarely less than two minutes. They are not easily converted to indicate the near-instantaneous variations in all the rain affecting a radio transmission path. Investigations are therefore in progress in many countries to collect new information relevant to the transmission problem and to find ways of utilizing the long-term records.

One method of acquiring information is to use the Sun as a source of millimetre radio-waves and to measure the attenuation caused by rain in the path. Statistical distributions of the probability that the attenuation will exceed specific levels may be obtained at different frequencies, but measurements at one frequency or at separate frequencies at different times do not give the variations of rainfall intensity along the path uniquely. Extrapolation to other frequencies is doubtful and the sizes of the rain cells are not directly deducible.

In the article by Hogg on page 337 of this issue of *Nature*, simultaneous measurements of attenuation at 16 GHz and 30 GHz on Earth-space paths using a Sun-tracker in New Jersey provide statistical estimates of the intensity and extent of rain showers during the daytime periods of one year. The same antenna was used for reception at both frequencies so that the attenuations at each frequency at any time arose from the same rain shower. The absolute attenuations and the corresponding ratio of the attenuations at each frequency were known for any percentage of the total observing time. The

ratio of the attenuation at 30 GHz to that at 16 GHz varied with the value of attenuation at 16 GHz. Assuming that the rate of rainfall was constant over part of the path and that the distribution of the raindrop sizes was the commonly-used one of Laws and Parsons, the attenuation ratio was calculated from theory for a range of rainfall rates and found to vary in a similar way to the variation of the ratio found experimentally. It was thus possible to relate the apparent rainfall rate occurring on the path to the attenuation at 16 GHz for any percentage of the recording time. As the attenuation coefficient is known theoretically, the path length through the rain at a specific apparent rate was deduced from the absolute value of attenuation.

In this way a statistical relationship was obtained between the apparent rain rate on the path and the extent of the rain for any probability level. For New Jersey, cell sizes ranged from about 11 km at rates of 5 mm h⁻¹ to less than 2 km at a rate of 100 mm h⁻¹.

The method enables observed statistics of attenuation to be extrapolated to give statistics at any other frequency where the assumed distribution of raindrop sizes is acceptable. And the information on the dimensions of rain cells is valuable for estimating suitable distances between earth terminals in path-diversity systems where switching between paths is used to avoid regions of rain producing large attenuations. This double frequency technique will also be suitable for communications on terrestrial paths.

From a Correspondent

ANIMAL BEHAVIOUR

Stress in Farm Animals

from a Correspondent

ALTHOUGH there is widespread public concern about possible stress and suffering in farm animals that may be caused by some husbandry practices, formulation of satisfactory legislation is hampered by an important unanswered problem. Is it possible to give an objective definition of suffering in animals? A symposium organized jointly by the Society for Veterinary Ethology and the Royal Society for the Prevention of Cruelty to Animals at the Zoological Society of London on May 25 and 26 considered the whole problem of defining and diagnosing stress in farm animals.

Professor J. R. Napier (Birkbeck

PULSARS

The First Century

JODRELL BANK announced last week the hundredth pulsar. Code-named PSR 1831-04 (the numbers being an abbreviated form of its location in the sky), it was discovered during a survey to examine the distribution of pulsars in the Galaxy, in particular to see whether the distribution is as expected if pulsars are indeed formed in supernova explosions. With thirty-eight pulsars attributed to them, the Jodrell Bank group are the most prolific discoverers of pulsars, and they are particularly proud that the instrument which they use, the Mark I dish, is the smallest telescope to have found one.

College, London), speaking on behalf of the RSPCA, opened the symposium by suggesting that although ethological studies must ultimately be scientific and objective, a little cautious anthropomorphism could with benefit be used as an interim measure, to give hypotheses as to what other animals feel.

No unified or simple definition of stress emerged from the meeting, but several speakers agreed that there may be two sorts of stress. There are short-term reactions to alarming or dangerous stimuli which are a normal part of an animal's behavioural repertoire and which are part of its adaptive mechanism for self-preservation. For example, fear may help an animal avoid being eaten by a predator. But apart from these normal reactions, there may also be continuing and severe manifestations of stress which are abnormal and harmful.

Some physiological measures of responses to unusual or damaging stimuli were discussed as indicators of either long-term or short-term stress—heart rate, electrical activity of the brain or overt manifestation of disease—but there was general acknowledgment of the difficulties of relying on these measures. Physiological and behavioural measures of stress do not always go together and there may be important differences in the manifestations of stress not just between mammals and birds but between different strains and even individuals. Indeed, it may transpire that no one measure of stress is sufficient and that a totality of symptoms should be taken into consideration.

It was evident from the meeting that knowledge of the behaviour of domestic animals is inadequate. Man is faced with the choice of regarding them simply as producers of eggs and meat or of acknowledging that they, like

human beings, have been shaped by natural selection to behave and to feel in certain ways.

BIOLUMINESCENCE

Identified Glow

from our Molecular Biology Correspondent

"I CAN think of nothing eerier/Than flying around with an unidentified glow on a person's posterior." Thus Ogden Nash, on the subject of the firefly. Little, one supposes, did he guess that the glow arises from the oxidation of the benzothiazole derivative, luciferin, by molecular oxygen after it has been bound to the enzyme luciferase and then adenylated by ATP and magnesium. This much is now established, and it is also known that the dehydrogenated derivative, which contains one more double bond, dehydroluciferin, will compete with luciferin, and undergo the same chemistry, but for the last step, the enzyme-bound adenylate not being reactive to oxygen. It undergoes in the course of binding some interesting and revealing changes in fluorescence, and to see more deeply into the nature of the interaction of luciferin with the enzyme, Bowie, Horak and De Luca (*Biochemistry*, **12**, 1845; 1973) have now synthesized a further analogue, which does not react with ATP. This is the alcohol, in which the carboxyl group of dehydroluciferin is replaced by a hydroxymethyl group, and is referred to as dehydroluciferol. Its spectroscopic properties are similar to those of its analogues, and it binds with the identical stoichiometry and a similar association constant to luciferase. In the bound state it is unaffected by ATP. Reciprocal plots show strong inhibition of the luciferase reaction.

In the accompanying article, Bowie *et al.* (*ibid.*, 1852) examine the nature of its interaction with the enzyme. In the first place, it must be noted that the fluorescence spectrum of luciferin and its analogues in aqueous solution shows a maximum in the region of 550 nm, which is characteristic of the phenolate form, in which the aromatic hydroxyl group is ionized. The excitation spectrum, however, is that of the neutral unionized molecule. This situation is not unique, for acids tend to become stronger on electronic excitation, and the proton can then dissociate during the lifetime of the excited state, leading to emission from the ionized species. When, however, the molecule is bound to the luciferase, a second smaller maximum, at about 425 nm, appears in the fluorescence spectrum, which arises from the unionized form. Thus the loss of the proton in the excited state is inhibited by the environment of the active site. There is at the same

time a blue-shift in the phenolate emission, and both these phenomena are easily enough interpreted in terms of the location of the chromophore in a hydrophobic cavity. When now ATP with magnesium is added, a large increase in the relative fluorescent intensity at 425 nm ensues, indicative of a change in the character of the binding site, or of an increase in the fraction of ligand bound. In fact it turns out that both factors contribute, for titration in the presence of ATP and the corresponding binding plot show that the binding constant in these conditions is up by a factor of ten, but the resulting increase in the proportion of bound ligand is insufficient to account for the intensity change. The conclusion is that the quantum yield of the fluorescence of the bound form is increased by a factor of two or more.

Bowie *et al.* have also tried the effect of AMP, which is known to stimulate the chemiluminescence. It leads to a blue-shift in the phenolate emission, and the phenol peak is totally expunged. The polarity of the site can be matched by a solvent of low dielectric constant, chloroform, in which the phenolate emission of dehydroluciferol, which can be provoked in this medium by addition of a base to act as a proton acceptor, appears at the same wavelength. The AMP evidently also causes a change in the location of interacting groups in the binding site, in such a sense as to facilitate the proton transfer process. To complete the picture, the fluorescence decay kinetics have been observed. The initial formation of the unionized excited state is followed in the first few nanoseconds by the appearance of the excited phenolate. The proton transfer rate is

diminished in the luciferase complex, and still more in the presence of ATP. These rates are reflected in the phenol excited state lifetime, which is increased on ATP binding, but marginally decreased by AMP. The authors suggest that the very high quantum yield of the bioluminescence may well involve the protection of the emitting species from quenching processes, and from hydrolytic destruction, by the hydrophobic milieu.

Bioluminescence of course occurs not only in fireflies but also in some bacteria, fungi and various creatures of the deep. In some of the luminescent coelenterates the mechanism is more or less similar to the firefly, with oxidation of luciferin on luciferase by molecular oxygen. This occurs, for example, in the class Anthozoa. The complicating feature of this system, however, has been that the chemiluminescent emission *in vitro* occurs at 490 nm whereas the intact animal lights up green, with a maximum at 509 nm, and some vibrational structure around 540 nm. An explanation has been contrived in terms of energy transfer to another chromophore, and indeed the search for such an acceptor led to the isolation of a green-fluorescing protein. Anderson and Cormier (*J. Biol. Chem.*, **248**, 2937; 1973) reasoned that if the excitation transfer is to proceed with high efficiency, the luciferase and the fluorescent protein should be packaged together at high concentration. The supernatant of homogenates was then discovered to produce the 490 nm luminescence, whereas the pelleted fraction emitted at 509 nm. This particulate matter could be purified by sucrose gradient centrifugation, and was found to contain luciferase, green-fluorescent

Membrane Properties of Tumour Cells

IN *Nature New Biology* next Wednesday (June 13) Nicolson enlarges on the differences in agglutinating ability between normal and transformed cells. It has been known for some years that a number of plant lectins, which specifically bind certain saccharides, will agglutinate transformed but not normal cells.

This was attributed at the outset to the exposure of "cryptic" binding sites in the process of transformation. Since then evidence has accumulated that agglutinability of the cells is determined by the ease with which the glycoprotein receptors are able to diffuse in the plane of the membrane, and create clusters that promote strong interaction with another cell.

This cluster formation can be observed by labelling with ferritin-bound or fluorescent anti-lectin antibodies. It has also been reported that tumour cells

will agglutinate with concanavalin A, a typical lectin, at room temperature, but not at low temperatures. It would be expected that this effect results from a dependence of the fluidity of the membrane on the temperature, which is what Nicolson has now demonstrated.

Concanavalin A-treated cells labelled with fluorescent anti-concanavalin antibodies at 0° C show uniform surface fluorescence. Normal cells first incubated at 22 or 37° C and then cooled to 0° C look similar. On the other hand transformed cells treated in this way show fluorescent patches. Labelling at 0° C after fixation with formaldehyde inhibits the temperature-induced formation of clusters. Thus the distinctive behaviour of tumour cells towards lectins is evidently a function of a greater fluidity in the membrane bilayer, and this effect is not expressed at low temperature.

protein and all the luciferin sulphokinase, an enzyme responsible for mobilizing the luciferin from its stored sulphate derivative. There is also a calcium-triggered "photoprotein", representing another chemically related luminescent system. The luciferin and green-fluorescing protein are not easily extracted from the pellet and may well be associated with a membrane. The particles in the electron microscope do indeed show evidence of a unit membrane envelope, and are about 0.2μ across. They emit under the action of oxygen and calcium ions, and the kinetics of emission are described. Anderson and Cormier have termed their particles lumisomes, and have found them also in the other coelenterates that they have examined.

Luminescent bacteria also possess a luciferase, but the substrate in this case is reduced FMN, and an aliphatic aldehyde is also implicated. As in a large part of the bioluminescence field, the outlines of the chemistry have been defined by Hastings and his associates. Some of the properties of the interactions of FMN with the enzyme have now been studied by Meighen and MacKenzie (*Biochemistry*, **12**, 1482; 1973). They have shown that a negative charge on the flavine is essential, and that its distance from the ring is critical for the interaction. The binding site for this charge can be satisfied by a phosphate ion, which competes against reduced FMN. With a neutral flavine the phosphate, or other suitable anion, strongly stimulates the luminescence. The deduction then is that the binding of the anion at a specific site on the enzyme stabilizes a functional intermediate, the chemical nature of which has been inferred.

Hunting the Helix. Following my remarks last month about the relation between conformational characteristics and sequences in globular proteins, a reader has commented that the observations of Crawford *et al.* (*Proc. US Nat. Acad. Sci.*, **70**, 538; 1973) on hairpin turns were largely prefigured by Kuntz (*J. Amer. Chem. Soc.*, **94**, 4009; 1972), who also found that they occurred almost exclusively on the surface. This greatly increases the stringency of the turn as a structural constraint, and thus its predictive value.

Correction

IN the editorial "Knack or Knowledge?" (*Nature*, **242**, 494; 1973), the reference to Peart and Newman in the first paragraph on page 495 should be to the Institute of Physics Conference Series No. 16. The title of this book is *Radiation Damage and Defects in Semiconductors*.

ECOPHYSIOLOGY

Arctic Adaptations

from our Plant Ecology Correspondent

ONE of the aims of the plant ecologist is to be able to explain the geographical distribution of plant species in terms of their adaptation to climatic conditions. Particular emphasis has been placed recently upon the temperature requirements of enzymatic systems within the plant, especially those enzymes associated with carbon dioxide fixation. Photosynthetic rates are known to be temperature sensitive and it has been demonstrated by Wareing *et al.* (*Nature*, **220**, 453; 1968) that the activity of ribulose-1,5-diphosphate (RuDP) carboxylase is an important controlling factor of this activity in light saturated conditions. It is natural that this enzyme has received considerable attention from ecophysiologists.

Treharne and Eagles (*Photosynthetic*, **4**, 107; 1970) found a higher rate of activity at low temperature for this enzyme in Norwegian populations of the grass *Dactylis glomerata* than in those from Portugal. Similar results have been obtained with conifers along altitudinal gradients (see *Nature New Biology*, **239**, 65; 1972). Recent studies on several plant species from cold habitats, however, suggest that the response of photosynthetic rates to tem-

perature is not always mirrored by the response of RuDP carboxylase activity.

Chabot, Chabot and Billings (*Photosynthetic*, **6**, 364; 1972) have found that populations of the Arctic-alpine species *Oxyria digyna* from Arctic environments (76° N) and from high altitudes in lower latitudes (37° N) all have RuDP carboxylase with temperature optima in excess of 45° C. The observed decrease in photosynthetic rate in this species at temperatures above 25° C cannot therefore be explained in terms of a falling activity on the part of the carboxylating enzyme.

Tieszen and Sigurdson (*Arctic Alpine Res.*, **5**, 59; 1973) have now come to similar conclusions when working with three Arctic grasses, *Alopecurus alpinus*, *Arctagrostis latifolia* and *Dupontia fischeri*, collected near Barrow, Alaska; in all of these species RuDP carboxylase activity was found to rise with temperature, reaching an optimum at 50° C. As in *Oxyria*, the photosynthetic rates of these grasses decline above a temperature of 25° C or less, and indeed the optimum temperature for their RuDP carboxylase activity is above their temperature compensation points.

The adaptation of photosynthetic mechanisms to environmental temperature conditions is evidently far more complex than can be explained by reference to the response of a single enzyme. Once again one must turn to other

Transformed Cells Express More of their DNA

It has long been suspected that transformed cells, being extremely active metabolically compared with normal cells, would have a greater proportion of their genome open for transcription. Early estimates of the amount of DNA transcribed were obtained using hybridization conditions which provide information on transcription of the repetitive fraction of the genome only. In *Nature New Biology* next Wednesday (June 13), Grady and Campbell report some careful hybridization studies which give information about non-repetitive DNA transcription in normal and transformed mouse cells grown in tissue culture.

Two normal cell lines, AL/N and Balb 3T3, were compared with their virus transformed derivatives PY AL/N and SVT2. Nonrepetitive DNA was isolated under the standard conditions of 60° C, 0.12 M phosphate buffer using only the fraction of DNA which renatured at a $C_0t > 220$ and was shown to be essentially free of repeated DNA. RNA-DNA saturation hybridization experiments were evaluated by the double reciprocal plot method of Bishop *et al.* and the saturation values obtained were thus shown to be independent of the RNA concentration used.

Approximately 20% of the nonrepeti-

tive DNA in normal AL/N cells is expressed in confluent (cells grown to their maximum cell density) and subconfluent cultures. When a mixture of confluent and subconfluent AL/N RNA was used it was found that the unique DNA sequences expressed in these two growth stages differed by as much as 5%. Saturation hybridization experiments with PY AL/N RNA showed that in virus transformed cells 30% of the unique DNA sequences is expressed in both confluent and subconfluent cultures. In this case, however, the regions of unique DNA transcribed in the two stages of growth were identical. In Balb 3T3 and SVT2 cell lines the amount of unique DNA sequences transcribed is again 20 and 30% respectively, in spite of large differences in chromosome number among all four cell lines.

Mixing experiments showed that the two normal cell lines differed by as much as 3% in the nonrepetitive DNA expressed and for the two transformed lines the difference was as much as 7%.

Grady and Campbell have thus presented firm evidence that virus transformed cells express more of their unique DNA and that the new sequences which are transcribed may vary from one transformed line to another.

factors, such as respiratory mechanisms and leaf resistance to the diffusion of carbon dioxide, for an answer to these seeming anomalies.

VIROLOGY

Sudden Death in Infancy

from a Correspondent

THE very sudden and unexpected death of a previously healthy infant—the so-called cot death—occurs more frequently than deaths in childhood due to road accidents. Yet the true cause of these cot deaths is rarely established although several theories are being examined. It may be caused by an overwhelming virus infection, or an allergic reaction possibly to regurgitated milk entering the trachea, or it may simply be that very young infants do not adapt sufficiently quickly to mouth breathing when their nasal passages are blocked.

A group of workers from Newcastle and Gateshead have studied fifty-one cases of sudden and unexpected deaths in infants (Ferris *et al.*, *Brit. Med. J.*, 2, 439; 1973). They were looking for a link between virus infections of the respiratory tract and sudden death in infants under a year old, and therefore excluded all infants known to have been unwell before their deaths, those with any obvious defects such as congenital heart disease and those who at post-mortem were found to have any disease which could account for their death.

Their investigation of the fifty-one remaining unexplained deaths involved histological examination of lung tissue and attempts at virus isolation and identification. The viruses looked for were those known to produce respiratory illnesses in children—respiratory syncytial virus, influenza A, and parainfluenza types 1 and 3.

Each slide of lung tissue was examined by two pathologists independently and complete agreement reached on their classification. The samples fell into three groups: the largest group of thirty-three cases showed changes of a type which have been widely accepted as characteristic of cot deaths, the main feature being a large volume of fluid in the alveoli of the lungs. These lung changes are similar to those found in children whose cause of death was thought to have been an acute allergic reaction. But in the Newcastle cases there seem to have been no obvious factors which could have provoked such reactions. No viruses were isolated from this group.

The second largest group contained sixteen infants whose lungs showed a breakdown in the lining of the smallest bronchial tubes and infiltration of lymphocytes. Viruses were isolated

from thirteen of these cases, chiefly the respiratory syncytial virus.

Group three showed the appearances of bronchopneumonia, a bacterial infection. There were three children in this group, from two of which respiratory viruses were isolated.

So the chief point is that in a third of these cases of cot death viruses were isolated and seem to have been the cause of death because the viruses were not of the type expected in the lungs of healthy children. But clearly this is not the whole story, for it looks as if some kind of unexplained allergic reaction is involved in a proportion of cot deaths. Physicians are, however, no nearer a way of anticipating and preventing these tragic sudden deaths.

MARS

Mariner Results

from a Correspondent

THE Royal Society discussion meeting arranged by the British National Committee on Space Research on May 15 and 16 showed how drastically Mariner 9 has changed our view of Mars. Mariners 6 and 7 saw a cratered planet like the Moon except for some evidence of erosion, but Mars is now seen to be much more varied and to have many features in common with Earth.

A preliminary geological map of the whole planet, prepared from Mariner 9 photographs, is now available. Only half of the surface, mostly in the southern hemisphere, is substantially cratered. The surface in the south is more than 3 km higher than in the

north and it is deduced that in the southern half of Mars the crust is ancient, low density, "continental" material. Thus about half of the surface of Mars is "continent", making it intermediate between Earth and Moon. It may be that the fraction of continental material might be a measure of the crustal stability of a planet.

It follows that martian material is not of uniform composition and so it seems that, like Earth and Moon, differentiation occurred early in the planet's history. In support of this view, spectral analysis shows the atmospheric dust to be 60% silica, and some very ancient volcanoes can be seen.

Volcanic activity that is geologically more recent covers a large area, and several volcanoes are similar to terrestrial shield volcanoes. The largest, Nix Olympica, is 700 km across and more than 24 km high. Three smaller shield volcanoes are aligned along the edge of the "continent". In the same region a vast canyon up to 6 km deep, 200 km wide and about 3,000 km long dissects the highest part of Mars. These observations suggest that some recent crustal movement of the type seen on Earth has occurred.

The entire surface has been modified by erosion and the most effective agent is wind. The martian atmosphere, almost wholly carbon dioxide, circulates due to differential solar heating, but it is only 0.5% as dense as Earth's atmosphere and so allows sharper temperature gradients to develop, resulting in generally stronger winds. Occasionally the winds become so strong that the lower atmosphere is filled with dust, although

Archival Records Hint at Nature of OH471

AS expected, the discovery that the QSO OH471 has a redshift of 3.4 has caused a flurry of activity among observational astronomers. In *Nature Physical Science* next Monday (June 11), Kraus, Gearhart and Ehman present flux density data for the object going back to 1963, while in this issue of *Nature* (page 336) Wampler and colleagues report that they have found a redshift of 3.53 for the "blue stellar object" OQ172.

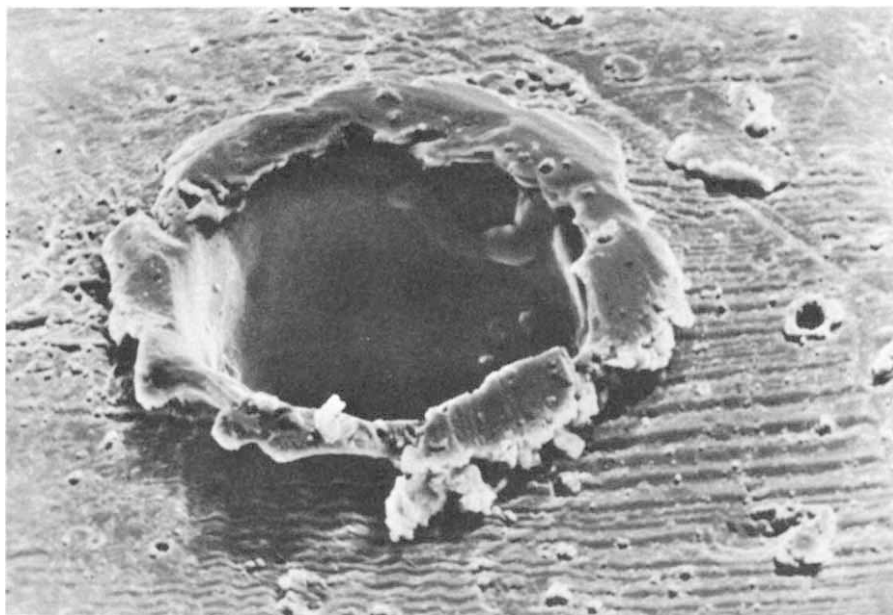
Clearly, the discovery of one high redshift object has indeed paved the way for other similar discoveries. But what of the observations of OH471 going back over ten years? It is certainly an almost unique event in radio astronomy that records of this kind have immediately been available for an object of newly discovered significance, although in the case of optical astronomy objects such as 3C273 can be examined on plates dating back to the latter part of the nineteenth century. The relevant radio data have been preserved, together with

other data from many objects, in the Ohio State University Radio Observatory archives.

It is only a pity that such foresight has not been rewarded with more dramatic discoveries than that OH471 is not variable. The flux density has been measured at 1,415 MHz, 612 MHz and 2,650 MHz. With a density of about 2 f.u. at the lower frequencies and 1 f.u. at 2,650 MHz and above, the spectrum is of the centimetre excess (CE) type, and might have been expected to show variability.

The archival records dash these hopes; the range of the (averaged) flux densities is only from 3.2 ± 1.2 f.u. to 2.09 ± 0.6 f.u., which can be interpreted as indicating either no significant change or a slight decrease over the period 1963–70. Although this is slightly disappointing, the availability of these records will ensure that any variation of the source will become apparent much more quickly than it would if monitoring was only now beginning.

SELENOLOGY

Microcrater in Lunar Meteorite

This lunar crater is only three hundredths of a millimetre across. It was found in a 2 mm by 1 mm fragment of nickel-iron brought back from the Moon by the Apollo 16 astronauts. This specimen is part of a sample sent to the Max-Planck Institute for Nuclear Physics at Heidelberg, and the photograph was taken with a scanning electron microscope at the Siemens laboratory in Karlsruhe. The specimen is itself a fragment of meteorite and has in turn been struck by a micrometeorite which must have been travelling at more than 10 km s^{-1} . As well as the main crater the impact has produced a series of parallel structures in the surface of the sample, together with a smaller crater to the right of the main crater.

it is not known exactly how this happens. The dust storm in progress when Mariner 9 reached Mars became the longest and most severe ever seen. When the dust eventually settled into a layer possibly only a few millimetres thick the effects of aeolian action were seen everywhere. Slides were shown of features formed by wind in terrestrial deserts.

Craters interrupt the smooth flow of wind, causing the formation of dunes inside craters and long streaks downwind of them, and an analysis of streak directions has allowed the atmospheric circulations to be plotted, although only the abnormal conditions of a planet-wide dust storm may be represented. The importance of aeolian effects on Mars has stimulated modelling of erosion features in wind tunnels in the hope that spacecraft photographs may be more fully interpreted. Films of the erosion of craters in a wind tunnel were shown and obvious similarities with martian craters were seen.

Wind cannot account for all the erosion, however. In particular, channels are seen, some of them hundreds of kilometres long, which are morphologically similar to intermittent streams in terrestrial deserts. The meeting participants accepted that this implied the existence of huge quantities of flowing water at some time although water on Mars would quickly evaporate. A clue to the origin of these channels is the

observation of areas of "cracked ground", characteristic of permafrost regions, near the heads of some of them. It seems possible that rapid melting of

permafrost could have given rise to transient rivers but the origin of the permafrost is unknown.

Extreme dryness is the most serious impediment to the development of life on Mars. Consequently the two landing craft of the 1976 Viking mission, whose main object is the search for life, will be put down where water is most likely to have been present. One will land at a confluence of dry river channels and the other in the region with most water vapour at the time.

The polar regions of Mars also received attention. At each pole there is a region of "laminated ground" which is interpreted as a 6 km thick covering of layered deposits which are at present being eroded. The material removed is being redeposited in the zones north and south of 30° latitude which are covered by a smooth mantle and appear to be areas of deposition. In spite of the thick layer of deposits the poles are lower than their surroundings and this means that isostatic adjustment—the sinking of the crust under the extra load—has occurred.

This and the volcanic evidence indicate that the interior is warm and the crust relatively thin so that the idea of incipient crustal movement is plausible. Evidence against this is that Mars is not in hydrostatic equilibrium—this long suspected fact has been confirmed by Mariner 9. This could imply great internal strength but it may be possible to account for it if the interior of Mars, like the Earth's mantle, is undergoing thermal convection.

Link between Coronal and Interplanetary Shocks

CORONAL shock waves generated by solar flares are directly linked to interplanetary shock waves in the solar wind, according to evidence gathered by Pintér and reported in next Monday's *Nature Physical Science* (June 11). This follows the now well established link between type II solar radio bursts and flare-generated coronal shock waves as another step towards a more complete understanding of the Sun's activity as an overall phenomenon rather than a series of unrelated events.

Coronal shock waves are emitted by flares at the time of the triggering instability which can be observed in hydrogen alpha light; at the same time, the type II burst is initiated. Radio data have shown that the resulting shock moves out to at least $7 R_\odot$, at a velocity of 500 to $4,000 \text{ km s}^{-1}$. At that distance from the Sun, the radio technique used becomes inadequate to monitor further expansion of the wave front. But satellite observations, notably from OSO-7, have now revealed the existence of plasma clouds, moving at velocities of around $1,000 \text{ km s}^{-1}$, at

distances beyond $10 R_\odot$ from the Sun. It now seems that such clouds represent coronal shocks which have escaped from the corona.

Pintér has shown the close relation between flare-generated coronal and interplanetary shock waves by comparing their velocities. Using two detectors (either two artificial space probes or one probe and Earth based instruments) at different distances from the Sun the velocities of the interplanetary shocks can be measured directly. All of those chosen for study were associated with type II bursts, and all were decelerating as they moved outwards. Extrapolating backwards, Pintér deduces the initial velocities of the shock waves, and finds that at $1 R_\odot$ these are almost identical to the velocities of the coronal shock waves associated with the equivalent type II bursts, measured from the drift of the burst.

For average values of Pintér's data—initial velocity $2,000 \text{ km s}^{-1}$, velocity at 1 a.u. 530 km s^{-1} and particle density 10 cm^{-3} —the energy supplied by the flare to the shock is $6 \times 10^{30} \text{ erg}$.

ASTRONOMY

Goddard Symposia

from Correspondents

THE Goddard Space Flight Center in Maryland was host recently to two international conferences: an X-ray astronomy symposium on April 27; and the first symposium and workshop devoted exclusively to gamma-ray astrophysics on April 27 to May 2.

X-ray Astronomy

Goddard seemed an appropriate place for the X-ray astronomy symposium because it was the project management centre for the first Small Astronomy Satellite (Uhuru) which has provided such a wealth of data on X-ray astronomy and has stimulated not only ideas for other X-ray experiments, but also interest in related measurements in optical and radio astronomy.

The first speaker at the morning session was Dr R. Giacconi (American Science and Engineering), the principal investigator of SAS-I, who began by announcing the publication of the third *Uhuru X-ray Source Catalog*. In addition to this catalogue, now containing a total of 161 X-ray sources, the position uncertainties for many of the sources previously listed in the second Uhuru X-ray catalogue have been significantly reduced. He described the large class of objects observed, including the binary systems. It now seems that the X-ray star of such binary pairs is sometimes quite massive and releases very large amounts of energy, and this has led to speculation that these particular objects are black holes.

Dr E. Kellogg (American Science and Engineering) described information on X-ray objects not in the galactic plane. He noted that the study of large clusters with redshifts of about 3 which are detectable in X-rays could give very deep insight into the evolution of galaxies.

Dr L. Peterson (University of California) noted that the energy spectra of X-rays associated with binary objects are often rather flat to 30 or 40 keV, at which energy the spectrum is observed to steepen sharply. No conclusion was reached regarding the most probable cause of this change in spectral shape.

Dr G. Garmire (CalTech), who concentrated on the soft X-rays, pointed out that nearly all classes of X-ray emitting objects discovered at higher energies by Uhuru are observed in the soft X-ray region. In general, soft X-ray observations can assist in determining distances to X-ray objects attributed to interstellar absorption. In addition, said Dr Garmire, future spectral studies of binary objects should lead to a detailed knowledge of the gas flow in these systems.

The afternoon session was devoted to the correlation of X-ray data with radio and optical observations and their relation to theoretical models for the sources. Dr R. Hjellming (National Radio Astronomy Observatory) spoke on radio emission from X-ray stars, Dr W. Liller (Harvard University) discussed the optical characteristics of X-ray sources and Dr K. Davidson (Princeton University) considered some theoretical aspects of X-ray source models, particularly for binaries. Although Sco X-1 is the brightest X-ray star in the sky, observed in the optical and radio bands as well, and the first to be discovered, Dr Davidson concluded that it cannot be explained in terms of a simple binary model as used for other X-ray stars. Hercules X-1, the source which is most nearly understood, is clearly a member of a binary system. The optical companion for this X-ray source has been well studied and its variations traced back about eight years by examining old Harvard plates. Other point X-ray sources exhibit less well behaved variability but have significant radio counterparts. Variations in the radio and X-ray bands have been correlated for the black-hole candidate Cygnus X-1. On the other hand, it was pointed out that the most pronounced radio variability observed, that for Cygnus X-3, did not correspond to any comparable variations in the X-ray band.

Gamma-ray Astrophysics

Important new results on the diffuse gamma-ray background as obtained by Apollo were presented by Drs L. Peterson (University of California, San Diego) and J. Trombka (NASA) and results obtained by SAS-II were presented by Dr D. Kniffen (NASA), who also reported observations of the galactic plane. The results from SAS-II confirm some important qualitative results first obtained by OSO-III that the Galaxy is an intense source of gamma radiation above 100 MeV and it stands out above the extragalactic background in this energy range. The spectrum is harder above 100 MeV than the gamma radiation seen at high galactic latitudes which is presumably extragalactic. The SAS-II results also indicate that the extragalactic (high galactic latitude) background spectrum is quite steep above 40 MeV.

Results from balloon observations by groups at the Max-Planck Institute and the US Naval Research Laboratory, reported by Dr G. Share (NRL), are consistent with the Apollo and SAS-II results which present a continuous observational spectrum from 300 keV up to 135 MeV. These data suggest a bulge in the gamma-ray spectrum above 1 MeV, in spite of background corrections which are of most importance below 4 MeV. This bulge has been

interpreted as a new component of gamma radiation at energies above 1 MeV. This argument is even more important if the X-rays below 1 MeV are thermally produced and are falling off exponentially in energy above 100 keV as was suggested by Drs D. Schwartz (American Science and Engineering) and R. Cowsik (University of California, Berkeley). Problems with the thermal interpretation were discussed by Dr W. Kraushaar (University of Wisconsin).

The interpretation of the 1 MeV to 100 MeV bulge in the gamma-ray spectra was discussed by Dr F. Stecker (NASA), who concluded that the excess is most likely caused by matter/antimatter annihilation. The Apollo and SAS-II observational data present an excellent fit to the predicted annihilation spectrum up to 135 MeV. The matter/antimatter-symmetric cosmology was discussed by Drs R. Omnes (Laboratory of Theoretical and High Energy Physics, Orsay) and E. Schatzman and J. Puget (Paris Observatory, Meudon).

The exciting aspects of the matter/antimatter cosmology reported on by Drs Omnes, Schatzman, Puget and Stecker indicate that, in addition to implying baryon symmetry on a universal scale, it can explain such diverse phenomena as: the cosmic gamma-ray background spectrum; the ratio of photons to nucleons in the Universe of $\sim 10^9$; annihilation as the energy source for generation of large-scale turbulence leading to galaxy formation; and the consequent observed sizes, mean densities and rotational velocities of galaxies.

The galactic gamma-ray flux in the 100 MeV range seen by SAS-II and OSO-III indicates an increase in the direction of the galactic centre. The most likely implication is that there is a cosmic-ray gradient toward the galactic centre, as was pointed out in remarks by Professor A. Wolfendale (University of Durham) and in a communication by Dr V. Ginzburg (Lebedev Institute, Moscow).

Another interesting highlight of the meeting was the discussion of the 470 keV feature seen at the region of the galactic centre. Three interpretations of this feature were presented. Professor D. Clayton (Rice University), who discussed gamma-ray line emission, mentioned that it may be caused by lithium. Dr R. Ramaty (NASA) (who, with Dr D. Forrest (New Hampshire), discussed gamma rays from solar flares) suggested that this feature could be attributed to redshifted positron annihilation produced at the surface of neutron stars. (This has also been suggested by Guthrie and Tidemaru in *Nature Physical Science*, **241**, 77; 1973.) Dr M. Leventhal (Bell Laboratories) suggested that this feature may arise from a poorly resolved positronium annihilation spectrum.

Prospects for Cancer Research in Britain

This article sets out the comments of the Joint Cancer Research Campaign/Medical Research Council/Imperial Cancer Research Fund Coordinating Committee for Cancer Research on Lord Zuckerman's recent report.

IN a preface to Lord Zuckerman's report on cancer research in Britain (HMSO, October 1972), the Prime Minister invited the comments of the Coordinating Committee for Cancer Research as well as of other bodies. The committee represents the Medical Research Council, the Cancer Research Campaign and the Imperial Cancer Research Fund. Its comments on the Zuckerman report, now with the government, are as follows.

The committee agrees that "money alone will not buy the new galvanizing ideas that are needed". Such ideas spring from original minds, but these minds need to have had appropriate training and a suitable environment in which to develop. Moreover, new ideas are produced against a background of accumulating basic knowledge which, at the present time, is coming from a wide spectrum of activities spanning the whole field of biomedical research.

There is no evidence that, nationally, Britain is short of intellectual potential; there are, however, inadequate opportunities to enable this potential to be brought to bear, and money is a limiting factor in providing these. If, therefore, it is desired to enlarge and accelerate the cancer research effort already going on in Britain, it is necessary to plan ahead for a long-term effort in terms of establishing more training and career posts for cancer research workers, providing new and better facilities for them, and ensuring, as the committee has already emphasized, that their work is carried on in close association with the main stream of other biomedical research.

Additional funds would enable a start to be made virtually at once in providing more training and career posts, but a limit would quickly be reached because of the lack of physical facilities, particularly accommodation. There is therefore a need to commit funds now to enable the planning of facilities to go ahead and, as these are provided, for example by the setting up of new oncological centres, so the number of posts should be further increased.

The committee makes two specific recommendations below about immediately foreseeable needs, that is, those on which it believes that money could actually and usefully be spent within a period of about two years from the time of commitment of new funds. Such a commitment would enable a start to be made in gearing up for an expanding programme. More funds would undoubtedly be needed beyond this initial period, but precisely how much is more difficult to forecast. This will depend on the degree of success in implementing the present proposals and on the development of new scientific leads from existing programmes. The committee, however, agrees with Lord Zuckerman that "a steady and substantial increase over the years" is likely to yield valuable results.

Senior Posts

The committee has already welcomed the decision of the Cancer Research Campaign to establish a number of new chairs of clinical oncology. It also takes the view that there is a need for the establishment of further consultant posts within the National Health Service for specialists in the cancer field. Some of these posts will undoubtedly be needed both where there are chairs of clinical oncology and in oncological centres. The committee also considers that some might be associated with major hospitals, especially teaching hospitals, throughout the country. In the first two years it might be appropriate to establish ten such new consultant posts. While initially many of these posts will be filled by clinicians trained in relevant specialities that are currently recognized within the Health Service, the committee hopes that in the longer term the necessary steps will be taken to establish oncology as a speciality in its own right, together with its own career structure.

There is also a need to establish a number of additional senior non-clinical posts with security of tenure. The committee envisages that in addition to being held in existing cancer research institutes, oncological centres, university departments and other institutes undertaking relevant basic research, a number of such posts should be tenable in clinical departments (including those in universities) where no career structure exists at present for those wishing to remain in a clinical environment. The committee believes that there is scope for the establishment of between eight and ten new posts of this kind at about senior lecturer level within the next two years.

Training Posts

The committee recommends that in the immediate future between twenty and thirty additional training posts should be provided for work in each of the disciplines already identified as being of particular relevance to cancer, that is, immunology, virology, epidemiology, developmental biology, mammalian cell genetics, and also in radiobiology. The majority of these posts might be held in university departments, but some should be in the cancer research institutes as well as in basic research institutes with training facilities such as the National Institute for Medical Research.

There is an urgent need to attract clinicians to specialize in oncology and the committee suggests that in the longer term it may be necessary to establish additional training posts at the registrar or senior registrar level within the National Health Service for this purpose. The consultant posts and chairs in oncology mentioned above would ensure that such doctors were entering a speciality with career prospects comparable to those in other disciplines. The number of training posts needed would depend on the number of consultant posts actually established and planned, and the number and quality of recruits coming forward to fill them.

Travelling fellowships are also needed for more senior workers and the committee recommends that five such fellowships should be allocated annually for this purpose.

If these proposals are accepted, there will be a need to finance supporting staff—technical staff, social workers, statistical and secretarial staff and so on.

Multi-centre Clinical Trials

The committee wishes to emphasize the importance of organizing more multi-centre trials, many of which will require a full-time medically qualified coordinator, and clerical and other assistance. In view of the rapid increase in the introduction of new methods of treatment it is recommended that four or five tenure posts should be established for this purpose together with supporting staff.

Clinical trials are useful not only in determining the effectiveness of new forms of treatment, for example, asparaginase and immunotherapy in leukaemia, but also in assessing the comparative benefits of combinations of different forms of therapy, for example surgery and radiotherapy. It is often necessary to conduct such trials on a multi-centre basis, because individual centres seldom have enough comparable cases within a short enough period. Although clinical trials may demonstrate only very marginal advantages of one form of treatment over another, they provide a certain way of making advances in the treatment of cancer and are also valuable when they demonstrate that a complex form of treatment which puts the patient to great inconvenience is unnecessary.

Screening Facilities

The committee envisages a need for the early commitment of funds for further research on the benefits of mass screening techniques. Pilot studies are not at the moment sufficiently far advanced for it to be possible to be precise in making recommendations, and any such projects would need to be discussed in detail with the Health Departments.

In general it may be said that the earlier the stage at which cancer is diagnosed the more amenable it will be to treatment, and the greater the chance of a clinical cure. In some instances special techniques can be used to demonstrate early malignant change and these methods are applied to a "normal" population in mass screening. To assess the potential contribution of such techniques, information has to be obtained on their sensitivity, specificity and acceptability, and large numbers of people may need to be examined and kept under observation for several years.

Facilities

The committee welcomes the decision to set up four new oncological centres in England on a trial basis. The Scottish Home and Health Department is currently considering the organization of oncological services in Scotland, in the light of a report received from the Standing Cancer Committee of the Scottish Health Services Council. This report advocates the development of the Beatson Institute for Cancer Research as the single Cancer Research Institute in Scotland and, if accepted, will undoubtedly lead to a requirement for further accommodation. Plans for an oncological centre in Wales, based in Cardiff, are under consideration by the Welsh Office.

The committee hopes that it will be possible to establish new research teams in these centres and recommends that funds should be earmarked for this purpose. If full advantage is to be taken of this opportunity to foster close liaison between clinical and basic research teams it will be essential to provide sufficient funds for the development of first-class facilities for patient care in these centres.

The committee strongly endorses Lord Zuckerman's view that accommodation for research could be improved. It has, in particular, identified the following as being likely to need an early injection of funds:

The Institute of Cancer Research exists at present on three widely separated sites (Fulham Road; Sutton, Surrey; and Chalfont St Peter, Bucks). There is an urgent need to rebuild the institute on one site at Sutton, Surrey. Plans are in hand which await only the necessary funds.

The Beatson Institute for Cancer Research in Glasgow. Plans for rebuilding the institute are far advanced, but although a grant of £360,000 has been awarded by the Wolfson Foundation a shortfall of about £100,000 in the total cost is anticipated during the next two years. A substantial further injection of funds will be required in the longer term.

Other opportunities for further expenditure include the support of a clinical unit associated with the new Tumour Biology Group at the MRC Laboratory of Molecular Biology at Cambridge; the development of facilities for trials of neutron therapy; the rehousing of the Cancer Epidemiology Unit at Oxford, now inadequately accommodated in three separate buildings; the provision of a new building for the MRC Mammalian Genome Unit at Edinburgh; the staffing and equipping of an experimental unit to work in parallel with the clinical research unit established by the ICRF at St Bartholomew's Hospital; and the rebuilding and modernization of the ICRF Laboratories at Mill Hill.

The committee is also investigating the possibility that the Microbial Research Establishment at Porton might do more work in relation to cancer.

Expanded Cancer Research Programme

The implementation of the specific proposals above will depend in the case of clinical and laboratory workers on the number of applicants of the requisite potential who in the event come forward. The committee believes that it should be possible to fill the posts specified within, say, two years of funds being made available. Some of the specific areas of research and projects suggested above are already under consideration by the sponsoring bodies. Others would need to be most thoroughly investigated before a final commitment was made. In many cases rapid decisions could be made, but in others preliminary work on the precise definition of the scientific programme, the recruitment of staff and provision of facilities would be required. Now, as always, when there is no single obviously correct path to follow, it will be important to ensure flexibility and to allow for the rapid switching of emphasis to take advantage of new leads. The committee accordingly recommends that a contingency fund of £100,000 should be made available annually as an uncommitted pool. Thus the committee has tried to put figures to its proposals and to indicate the possible shortfall between the present commitment of private and public funds, and what could be earmarked if it were desired to accelerate the pace of cancer research still further in Britain.

Table 1 Estimated Annual Costs

	Annual cost (£)
3 chairs of clinical oncology	81,000
10 NHS consultant posts	90,000
8-10 senior non-clinical posts	154,000
20-30 non-clinical training posts	80,000
6 clinical training posts	27,000
5 travelling fellowships	25,000
4-5 staff for clinical trials	25,000
Contingency fund	100,000
	582,000

The committee wishes to re-emphasize that any expenditure of funds on cancer research must continue to be the subject of the most stringent and uniform scientific scrutiny in order to ensure that the quality of work is maintained at the highest standard.

The costs of a development programme along these lines are estimated in the accompanying tables. Table 1 shows the

estimated annual cost of the opportunities listed above, and consists chiefly of the direct cost of salaries and staff support. It is important that the proposal for the establishment of chairs in clinical oncology is likely to be contained in the present plans of the Cancer Research Campaign. Much of the annual contingency fund would be needed by research teams associated with the oncological centres.

Table 2 Estimated Capital Costs

	Cost (£ million)
Institute of Cancer Research	3.50
Beatson Institute	0.80
ICRF, Mill Hill	1.00
Oncological centres	1.00
ICRF, St Bartholomew's	0.50
Clinical Tumour Biology Unit	0.80
Neutron therapy	1.00
Epidemiology Unit	0.08
Mammalian Genome Unit	0.22
	8.90

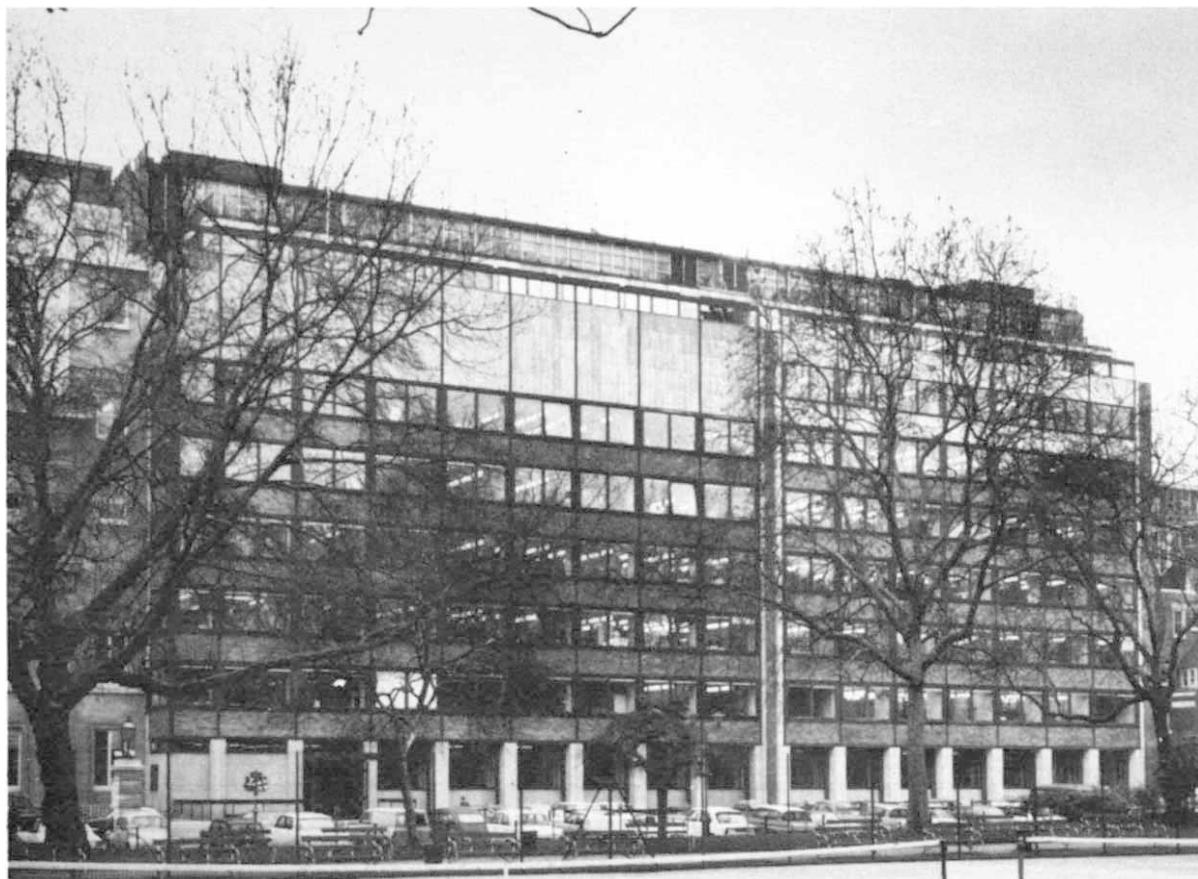
Provision for service aspects of oncological centres, family care, education and possible developments at MRE, Porton, is not included.

Table 2 shows the estimated capital cost of some of the development schemes listed above. Of the total capital cost of £8.9 million, £3.7 million would be needed in the first two years of an accelerated programme. Not all the cost would fall on the government—private sources, for example, are likely to contribute £250,000 to the rehousing of the Institute of Cancer Research, while the cost of developments such as the MRC Clinical Tumour Biology Unit is already included in the budgets of the departments concerned.

Allowing for arrangements of this kind already made, the total extra capital cost is likely to be of the order of £1.7 million in the first two years of an expanded programme.

Making similar allowances, £501,000 is the committee's best estimate of the maximum level of annual recurrent expenditure that might be reached towards the end of a two year period. There would be a gradual build-up to and beyond this level and the proportion that might be spent within the two years might be about £600,000. This figure, together with that of £1.7 million non-recurrent for building and facilities, is therefore the committee's best estimate of monies which if committed now might reasonably be expected to be usefully spent within the next two years on an expanded and accelerating national cancer research effort. It must, however, be emphasized that as far as recurrent expenditure is concerned the commitment should be regarded as long term, and that, in the case of capital expenditure, the greater part will be incurred after the initial two year period. The sums suggested will need to be reviewed annually and adjusted—almost certainly upwards—as programmes develop.

Earlier in this article the committee emphasized the point made in its first report that it was important that cancer research should not be carried out in isolation from the main stream of other biomedical research. Lord Zuckerman recognizes in his report that one of the effects of the American initiative will be to increase the pace of biomedical research. He has also commented that while fundamental research in this country may not hitherto have suffered from lack of funds, this situation may change in the near future. The committee agrees with these assessments. It thinks that it is of great importance to cancer research that there should be no diminution in the scale of the basic biomedical research effort already going on in Britain, and, in particular, that none of the proposals that they have made above should be implemented at the expense of other areas of biomedical research.



The Imperial Cancer Research Fund building in Lincoln's Inn Fields, London.

Palaeohydrology of Late Pleistocene Lake, Alexandersfontein, Kimberley, South Africa

KARL W. BUTZER

Departments of Anthropology and Geography, University of Chicago, Chicago, Illinois

G. J. FOCK

McGregor Memorial Museum, PO Box 316, Kimberley, South Africa

ROBERT STUCKENRATH

Smithsonian Radiation Biology Laboratory, Rockville, Maryland

A. ZILCH

Forschungs-Institut Senckenberg, 6-Frankfurt-am-Main

The closed Alexandersfontein depression, now only an evaporation pan, harboured a +19 m lake with a surface area of 44 km² and Middle Stone Age occupation, a little before 16,000 BP. Assuming a temperature depression of 6° C, calculations show that rainfall was about twice that of today.

CONTROVERSY prevails as to the nature of Pleistocene climates in the interior of southern Africa¹⁻⁴. For example, it is uncertain whether geomorphological⁵ and palynological^{6,7} indicators of relatively moist late Pleistocene climatic episodes reflect lower temperatures, higher rainfall, or both. In order to reduce some of the multiple, interacting factors that generally impede interpretation, a closed depression east of Kimberley was studied in 1971. Here a 328 km² basin drains ephemerally into the Alexandersfontein Pan, which lies athwart the Cape Province-Orange Free State border at approximately 28° 50' S and 24° 48' E. The floor of the pan receives a little spring influx but now only holds water sporadically after heavy rains; however, the depression once enclosed a number of substantial Pleistocene lakes.

The Alexandersfontein basin is formed on subhorizontal Dwyka shales extensively intruded by Karroo "dolerite" (diabase) sills, dykes and plugs that provide koppies of relief 30 to 100 m. Total elevation ranges from 1,119 m on the pan floor to 1,270 m along the irregular rim of the watershed. Whatever factors may have initiated the depression, one of countless such features in erosional topography around

Kimberley⁸⁻¹⁰, Pleistocene and later excavation must be attributed to periodic deflation, with crucial chemical weathering of the shales to which deepening is confined. The oldest surficial deposits are fragmentarily preserved, with little or no primary relief, along the eastern, southern and western margins of the depression (see Fig. 1). These consist of lacustrine and aeolian beds, often reworked, and reaching 55 to 60 m above the modern pan floor, although the present threshold of the basin is clearly defined at 1,151 m (+32 m), forming a conspicuous former overflow outlet to the Modder River drainage.

The younger lake beds under consideration here are shown in Fig. 1. They are better preserved and extensively exposed under a veneer of wash and drifting sand, and they seem to be confined by the 1,142 m (3,800 foot) contour of the 1:50,000 topographic maps (Nos. 2824 DB/DC/DD). Beach ridges of these younger lakes are well developed south of the modern pan (Fig. 1), with shoreline cliffs locally cut into shale along the northern peripheries. Specific stages are recorded at +17 to 19 m, +12 m and +6 m (see Fig. 2a and b).

Along section A (Fig. 2a), the highest lake beds reach +19 m and consist of a white, friable, "chalk": 81.5% CaCO₃, 3.5% opaline silica, and a further residue of silty clay. A sample of inorganic carbonate from a depth of 1 m gave a date of 16,010 ± 185 BP, with ¹³C = -0.77‰ (SI-1115). As there is no carbonate bedrock in the basin, except for localized surface calcretes, this age must be considered a minimum, although the silica structure, which does not break down in HCl, has presumably restricted recycling of the carbonates. The +12 m beds form more conspicuous ridges here, locally with a 2 m nip. One section (A, Fig. 2a) begins with a prismatic, brownish-grey, sandy silt (14.5% CaCO₃), which followed by a columnar, pale-brown silty sand (27% CaCO₃), by weakly stratified, yellowish-brown sandy clay (6% CaCO₃), and ultimately a coarse, reddish wash. Whereas the sands of the three lower beds are derived from breakdown of local shales and diabase,

the final wash includes subrounded, exotic quartz of aeolian type. Forms and deposits show that peripheral fluvial activity accompanied the +12 m lake stand, with the basal lacustrine beds succeeded by a lake-margin alluvium, and finally a fill, now exposed as a dissected fan.

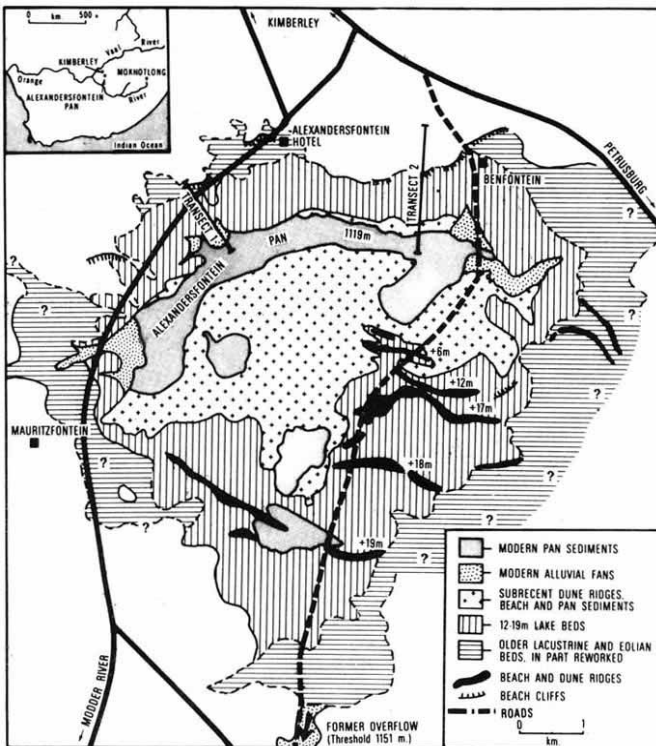


Fig. 1 Surficial deposits of the Alexandersfontein Pan.

Along section B (Fig. 2b) the +19 m lake is recorded by the upper half of a sequence that begins with a wash of silty sand (CaCO_3 sharply increasing from 7 to 27% at the top). Then follows a prismatic, light-grey marl (79% CaCO_3 , 6% amorphous silica) with a silty clay residue. The abundant small gastropods *Pupilla (Gibbulinopsis) fontana* (Küster), *Pupilla (Afripupilla) tetradus* (O. Boettger), *Vertigo antivertigo* (Draparnaud) and *Succinea striata* (Krauss) are terrestrial, but the last two are hygrophile, and we infer that these snails were reworked by rainwash or after a seasonal rise of lake level. A bulk sample of essentially inorganic carbonate gave a date of $11,025 \pm 110$ BP (SI-1116), with $\delta^{13}\text{C} = -0.44\%$. This is also a minimum date, but the secondary calcification and the presence of illuvial soil wash in small veins and hollows suggests a considerably greater degree of contamination by younger carbonates than in the case of SI-1115. These quiet-water, shoreline deposits are followed by a prismatic, light-grey clayey sand (9% CaCO_3 and 16% amorphous silica), and finally a similar silty sand (17% CaCO_3 , 20% opaline silica). Along an alternate section (see Fig. 2b), the final bed is overlain by 2 m of well-stratified, gritty alluvium that was truncated before a prismatic, dark-grey clay was laid down. These additional details suggest a temporary regression, accelerated runoff and alluvial influx, culminating in a transgression that cliffed the deposit, with the dark cracking clays probably laid down on the lake margin a little later. The sands are again local except for some rounded, exotic quartz in the penultimate bed, preceding the temporary regression.

Altogether the +17 to 19 m lake (with beach ridges or cliff bases at those levels, but undifferentiated deposits to +23 m

or higher) represents a long interval with appreciable oscillations of the shoreline, paralleled by persistent peripheral runoff and changes in the level of aeolian activity. The differential degree of "silicification" suggests a moderate to long time span before development of the +12 m shoreline, under similar conditions, with a last halt in regression at +6 m. Extensive deflation then attacked the centre of the basin, creating four separate pans of which the Alexandersfontein is by far the largest. Recent sedimentation involves a cracking, greyish-brown clayey silt, with 17 to 25% CaCO_3 , a trace of amorphous silica, and sands entirely of local origin (shale residues, feldspar, trace quartz); depending on the local importance of sodium salts, pH varies from 7.9 to 9.0. Other recent or sub-recent deposits (Fig. 1) include extensive, stepped aeolian beach ridges or lunettes, with traces of higher lake "muds" to +3 m (see Fig. 2b).

As two ^{14}C dates of a little over 17,000 yr were obtained from large aquatic snails in terminal high-floodplain deposits of the Vaal River northeast of Kimberley¹¹, we feel that the +17 to 19 m lake very probably has a date of about 16,000 BP or a few millennia before. It is beyond question contemporary with the maximum glacial advance in higher latitudes, in what has been designated the Würm Upper Pleniglacial¹², and *Vertigo* is a Palaearctic form. No materials suitable for dating have yet been recovered from the +12 m lake beds, but a Late Glacial age may apply here.

This is the type area where J. H. Powers collected the "Alexandersfontein variant" of the Middle Stone Age (MSA)¹³, and we found similar artefacts resting in profusion on the dark clays along transect b (Fig. 2), while Powers's collections came from wash on denuded surfaces of the +19 m lake beds or shorelines northeast of transect a (Fig. 2). A single, convergently-patterned, large Levallois flake on lydianite was found *in situ* well within a calcrete mantling the +12 m lake beds along transect a. Local MSA settlement was almost certainly coeval with the last stages of the +17 to 19 m lake, and may possibly have extended until (or resumed with) the +12 m lake. Corroboratively, D. M. Helgren and Leon Jacobson (personal communication) have recently discovered an MSA site with mammalian fauna *in situ* and at least partly in primary context in the 18 to 19 m shoreline south of the lake.

The palaeoclimatic implications of high, non-outlet lakes in the Alexandersfontein depression are considerable. The +17 to 19 m lake had an area of 44 km² and an average depth of almost 8 m, whereas the +12 m lake had an area of 24 km² and an average depth of 4 to 5 m. Evaporation over open water (by a Symons pan) now averages 2,120 mm yr⁻¹ in Kimberley¹⁴, whereas precipitation (averaged for nine stations in the quadrant 28° 50'–29° 00' S, 24° 45'–25° 00' E) is only 397 mm (ref. 15) with a calculated potential evapotranspiration of 932 mm, precluding all but incidental runoff from the drainage basin. In the upper Vaal drainage with almost double the rainfall and temperatures 2 to 3° C lower, the runoff quotient is only 9.3% or about 59 mm (ref. 16), implying that the calculated evapotranspiration by the Thornthwaite method here is 17.7% too high¹⁷. In the case of the +12 m lake, 1 mm of runoff across the entire basin would provide an influx equivalent to 11.7 mm of water over the 24 km² of lake surface, and in the case of the +19 m lake, 6.4 mm over a 44 km² lake. Consequently, assuming constant evaporation factors and no external groundwater losses or gains, and a reduction of potential evapotranspiration by 17.7%, the +12 m lake level could be maintained only with a precipitation of 1,046 mm and a runoff ratio of 0.9%.

It would be more realistic to assume a temperature depression of 6° C, a conservative estimate for the Würm Pleniglacial on the basis of periglacial and other cold-climate phenomena in South Africa^{18–20}. Recalculating the potential evapotranspiration, this gives 695 mm for Kimberley, which is identical to that in the upper Vaal drainage today¹⁷, so that the 9.3% runoff quotient can be safely used. Actual evaporation from open water exposed to a reduction in temperature of 6° C would be

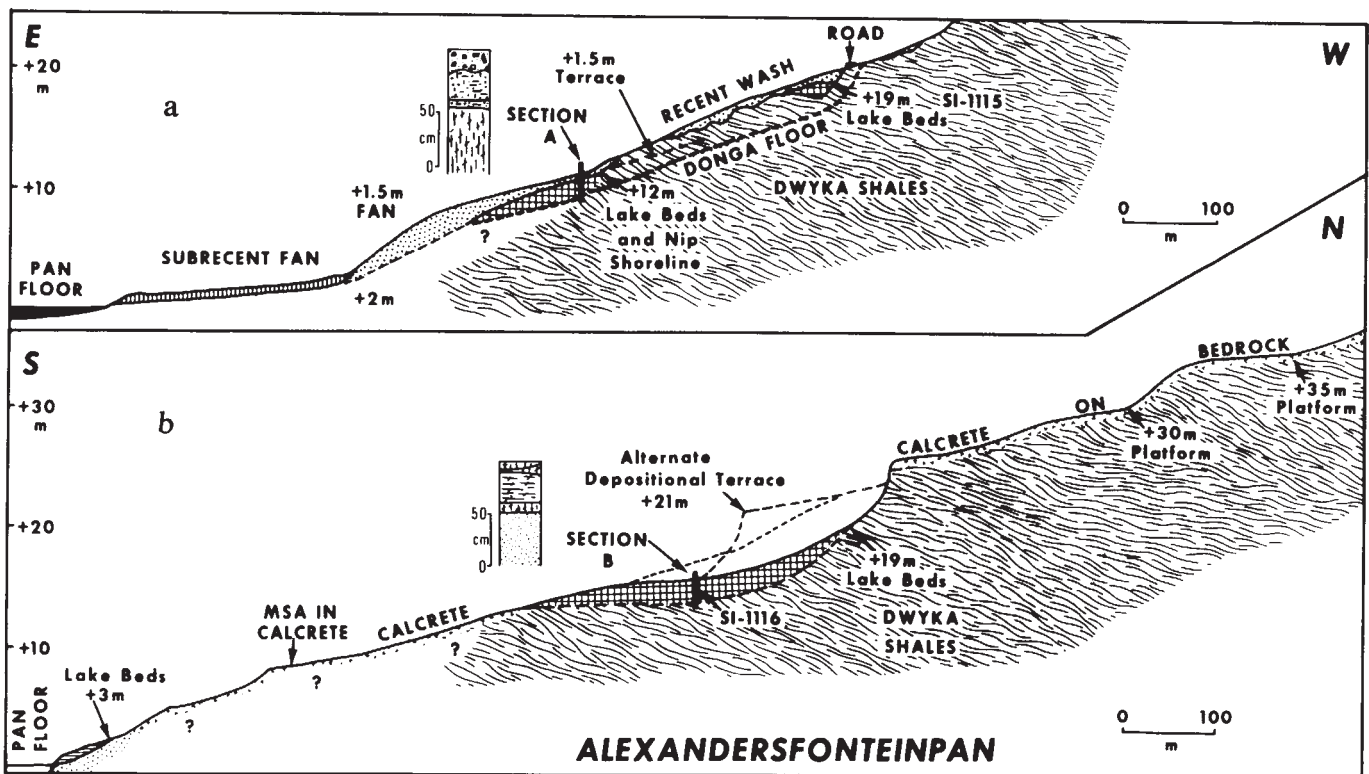


Fig. 2 a and b, Scale transects north of the Alexandersfontein Pan.

approximately 1,400 mm by analogy from the modern station Mokhotlong, Lesotho (12° C mean annual temperature compared with 18° C for modern Kimberley)²¹. In this case the hydrological budget would require 878 mm to balance with a +19 m lake and 671 mm in the case of the +12 m lake. By implication, with a 6° C drop of temperature, rainfall would theoretically need to be increased by 123% to maintain Pleniglacial Palaeolake Alexandersfontein at +19 m. The assumptions necessary to derive this figure caution against its use in more than a semi-quantitative manner. Even if temperatures and radiation received were substantially lower, however, rainfall would necessarily have been almost twice that of today.

Applying these data to a monthly lake budget, assuming a seasonal precipitation and evaporation regime analogous to that of today, and determining relative monthly distribution of runoff by a calibrated version of the Thornthwaite-Mather hydrological budget²², a seasonal fluctuation of 4.1 cm could be inferred for the +19 m lake, with a maximum level in May and a minimum in December. Such computations are, however, unrealistic, for it is necessary to assume seasonal changes of rainfall and radiation.

Nonetheless, the Pleniglacial climate of the Kimberley district was necessarily both substantially cooler and wetter than today—in a relative as well as absolute sense—so corroborating van Zinderen Bakker's interpretation of the Florisbad pollen profile⁹ and Coetzee's of that at Aliwal North⁷. This demonstrated wet period in the Interior Plateau of South Africa coincides with high lake levels in the middle latitudes but contrasts with a marked Pleniglacial dry phase in many parts of tropical Africa²³. Two meteorological interpretations are possible. Equatorial displacement of the zonal boundary between the southern circumpolar westerlies and tropical easterlies during a Pleniglacial circulation²⁴ would significantly increase winter precipitation, decrease summer rainfall. On the other hand, the northern hemisphere circulation may have been appreciably strengthened during the Pleniglacial, displacing the tropical circulations of Africa southwards and thereby

bringing the Kimberley region well within the summer rainfall belt (J. Bjerknes, personal communication).

Part of this work was supported by a grant from the National Science Foundation (to R. G. Klein and K. W. B.), as well as by the Anthropology and Geography Departments of the University of Chicago. The map and diagrams were drawn by D. B. Cargo.

Received October 30, 1972; revised February 20, 1973.

- ¹ Flint, R. F., *Bull. Geol. Soc. Amer.*, **70**, 343 (1959).
- ² Mason, R. J., *Prehistory of the Transvaal*, 35 (Witwatersrand Univ. Press, Johannesburg, 1962).
- ³ Bond, G., *Viking Fund Publ. Anthropol.*, **36**, 308 (1963).
- ⁴ Partridge, T. C., *S. Afr. Arch. Bull.*, **24**, 106 (1969).
- ⁵ Butzer, K. W., *Proc. Assoc. Amer. Geol.*, **3**, 41 (1971).
- ⁶ Van Zinderen Bakker, E. M., *Proc. Third Panafrican Congr. Prehist., Livingstone* 1955, 56 (1957).
- ⁷ Coetzee, J. A., *Palaeoecology of Africa*, **3**, 100 (1967).
- ⁸ Du Toit, A. L., *S. Afr. Geog. J.*, **16**, 3 (1933).
- ⁹ Van Eeden, O. R., *Tegnikon*, 206 (1955).
- ¹⁰ Butzer, K. W., *J. Arch. Sci.* (in the press).
- ¹¹ Butzer, K. W., Helgren, D. M., Fock, G. J., and Stuckenrath, R., *J. Geol.* (in the press).
- ¹² Van der Hammen, T., Maarleveld, G. C., Vogel, J. C., and Zagwijn, W. H., *Geol. Mijnbouw*, **46**, 79 (1967).
- ¹³ Goodwin, A. J. H., *Ann. S. Afr. Mus.*, **27**, 95 (1929).
- ¹⁴ *Climate of South Africa, Part 8: South African Weather Bureau, Pretoria, Mem.*, **28**, 227 (1965).
- ¹⁵ *Climate of South Africa, Part 2: South African Weather Bureau, Pretoria, Mem.*, **20**, 88, 104 (1954).
- ¹⁶ *Hydrographic Surv. Paper*, **6**, 1 (South African Irrigation Department, 1954).
- ¹⁷ Schulze, B. R., *S. Afr. Geog. J.*, **40**, 31 (1958).
- ¹⁸ Butzer, K. W., and Helgren, D. M., *Quat. Res.*, **2**, 143 (1972).
- ¹⁹ Butzer, K. W., *S. Afr. Arch. Bull.*, **28** (in the press).
- ²⁰ Butzer, K. W., *Boreas*, **2**, 1 (1973).
- ²¹ *Climate of South Africa, Part 8, op. cit.*, 79, 228.
- ²² Thornthwaite, C. W., and Mather, J. C., *The Water Balance (Publications in Climatology, 8, No. 1, Laboratory of Climatology, Centerton, NJ)*.
- ²³ Butzer, K. W., Isaac, G. L., Richardson, J. L., and Washbourn-Kamau, C. K., *Science*, **175**, 1069 (1972).
- ²⁴ Hastenrath, S., *S. Afr. J. Sci.*, **68**, 96 (1972).

Effects of Lysozyme on Normal and Transformed Mammalian Cells

E. F. OSSERMAN, M. KLOCKARS, J. HALPER
& R. E. FISCHER

The Institute of Cancer Research, Columbia University, and Department of Medicine, College of Physicians and Surgeons, 99 Fort Washington Avenue, New York City, New York 10032

Homologous mammalian lysozymes have specific effects on the *in vitro* growth patterns and morphology of normal and transformed mammalian cells, apparently due to effects on constituent membranes. Lysozyme may be important in the surveillance of membranes and may be a mediator of the antitumour functions of macrophages.

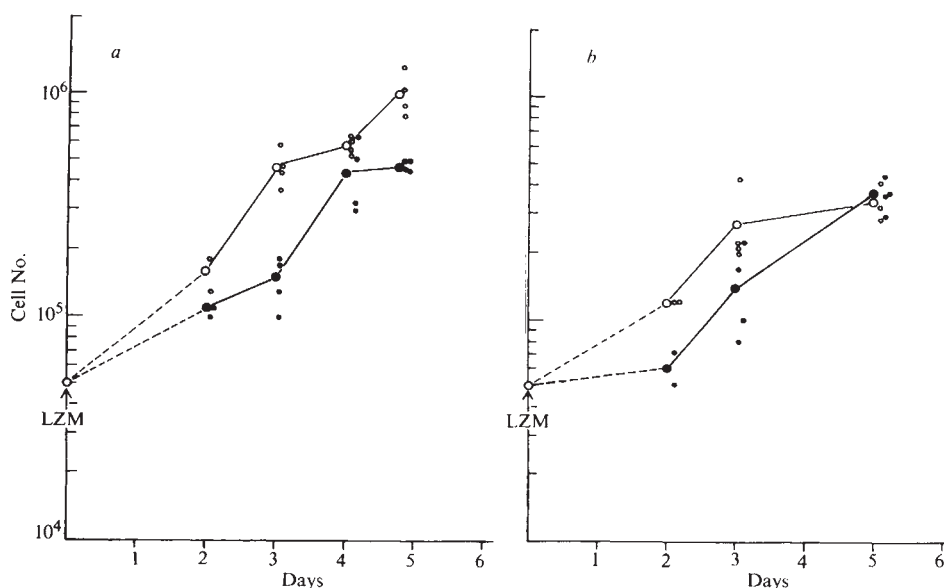
IN 1922, Fleming¹ reported a "remarkable bacteriolytic element found in tissues and secretions", which he designated "lysozyme" (LZM)—an enzyme capable of lysing (bacteria). Fleming's studies and other extensive investigations in the ensuing fifty years (see ref. 2 for a recent review) documented the widespread distribution of LZMs in animal and plant

tissues, elucidated their detailed structures, particularly those of hen egg white and human origin, and defined the precise mechanism of LZM's interaction with and cleavage of polysaccharides, chiefly those known to be constituents of bacterial cell walls. Fleming's concentration on the bacteriolytic actions of LZM and its role in antibacterial defence mechanisms probably limited speculation and investigation of possible non-antibacterial actions of LZM which may be of equal if not greater importance in mammalian physiology.

We have investigated the possibility that certain polysaccharides, glycoproteins or glycolipids of mammalian cells are susceptible to enzymatic cleavage by LZM. As these substances are major constituents of cell membranes, we further proposed that LZM may be important in the regulation of certain membrane-dependent cell functions and may participate in the surveillance of membrane abnormalities, particularly those associated with neoplastic transformation.

We examined the effects of LZM on the behaviour of normal and transformed murine and human cells *in vitro*, thereby seeking insight into the possible effects of endogenous LZM *in vivo*. We used homologous mammalian LZMs, that is LZM isolated from the urine of patients with monocytic

Fig. 1 Effect of rat LZM ($100 \mu\text{g ml}^{-1}$) on the proliferation of (a) normal (3T3) and (b) transformed (SV101-3T3) mouse embryo fibroblasts. The initial cell inoculum was 5×10^4 cells in a 5 cm diameter (Falcon plastic) culture plate, and LZM was present in the medium throughout the entire period of culture. $\circ$ and $\bullet$, Individual plate counts; $\bigcirc$ and $\bullet$, mean of each group. Black symbols, LZM; open symbols, control.



leukaemia³ and of rats bearing the transplantable chloro-leukaemia which elaborates LZM (ref. 4). Available evidence indicates that these LZMs are identical to their normal counterparts³. LZMs were isolated from urine by the bentonite adsorption method³. The purity of all preparations was ascertained by appropriate electrophoretic, chromatographic and immunological analyses.

Mouse Embryo Fibroblasts

Normal mouse embryo fibroblasts (3T3) and simian virus-transformed cells (supplied by Dr Claudio Basillco, New York University School of Medicine) were grown at 37° C in a 7% CO₂ : air atmosphere in Dulbecco's modified Eagle's medium (MEM) supplemented with 10% calf serum, penicillin (100 U ml⁻¹) and streptomycin (50 U ml⁻¹). In the experiments shown in Fig. 1, rat LZM (100 µg ml⁻¹) was added to the cultures at the time of initial plating. The initial cell inoculum was 5×10^4 cells in a 5 cm diameter culture plate. In these conditions of relatively low cell density and the presence of LZM throughout the culture period, the LZM appeared to effect a transient inhibition of the proliferation of both 3T3 and SV-3T3 cells, chiefly evident during the first 4 d in culture. At the fifth day, particularly with the SV-3T3 cells, there was no significant difference in the total cell numbers in the control and LZM-treated cultures. As Fig. 2 shows, however, there was a significant qualitative difference between the control and LZM-treated SV-3T3 cultures on the fifth day. Thus, in the control SV-3T3 cultures there was dense growth in the middle of the plate and sparse growth at the periphery, whereas the LZM-treated SV-3T3 cells were more uniformly distributed over the surface of the culture plate. Both the control and LZM-treated 3T3 cells were relatively uniformly distributed in their growth patterns. When rat LZM (100 µg ml⁻¹) was added to 3T3 and SV-3T3 cultures 48 h after the initial plating of 1×10^5 cells, that is twice the inoculum as in the study shown in Fig. 1, there was much less of an effect than in the previous experiments.

Fig. 3 shows the effects of increasing concentrations of rat LZM present from the time of initial plating on the uptake of ³H-thymidine by normal (3T3) and transformed (SV-3T3) mouse embryo fibroblasts cultured on hanging glass coverslips⁵. In both cell lines LZM produced a small but significant and apparently dose-dependent decrease in uptake. We also noted, particularly with the 3T3 cultures, that the LZM inhibition of ³H-thymidine uptake was most evident in the first 2 d *in vitro*, similar to the pattern observed in the cell counting studies.

Transformed Rat Liver Cells

Because LZM might alter different cells in different ways, we tested the effects of rat LZM on transformed rat liver cells. A cell line (RLB) which had apparently undergone spontaneous transformation when grown in a nutritionally-deficient medium was obtained from Dr Carmia Borek of the College of Physicians and Surgeons of Columbia University⁶. This line was maintained in RPMI 1640 medium supplemented with 5% foetal calf serum, penicillin (100 U ml⁻¹) and streptomycin (50 U ml⁻¹). The effects of rat LZM in concentrations up to 400 µg ml⁻¹ on the uptake of ³H-thymidine by these cells were essentially comparable with those seen with 3T3 and SV-3T3 cells. Some inhibition was evident during the first 2 d in culture, but by the third day there was no difference between the control and LZM-treated cells with respect to ³H-thymidine uptake. There were, however, significant cytological differences between the control and LZM-treated RLB cultures which were already evident after 2 d and were more marked after 4 d (Fig. 4). Whereas the control cultures exhibited a relatively uniform, epithelial hexagonal pattern, the LZM-treated cells showed very marked nuclear and cytoplasmic irregularities. Thus, the nuclei of the LZM-treated

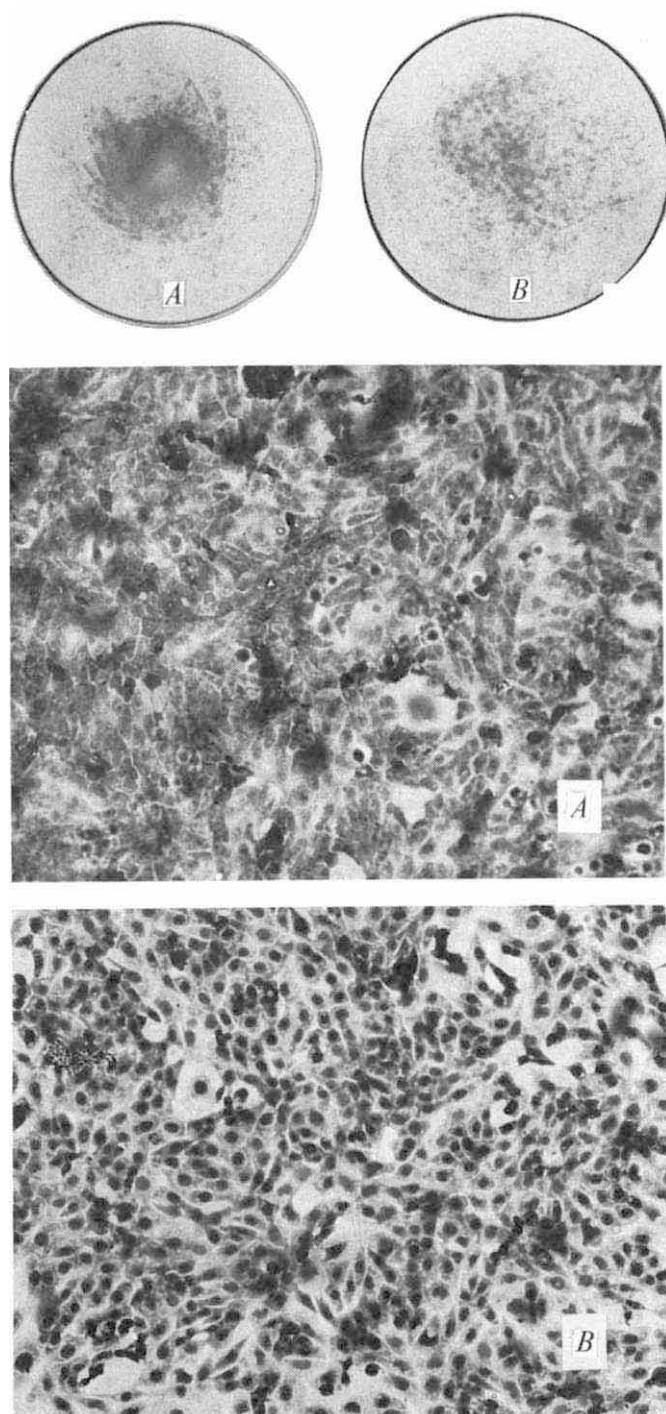


Fig. 2 Gross and microscopic appearance of SV101-3T3 cultures; (A) control and (B) grown in the presence of rat LZM 100 µg ml⁻¹, on day 5 of the experiment shown in Fig. 1. The LZM-treated culture shows a more uniform distribution on the plate and a more regular cytology with less pleomorphism.

cells varied considerably in size, shape and staining intensity, and many appeared distinctly pyknotic. The cytoplasmic changes in the LZM-treated cells consisted of marked spreading, development of surface invaginations and the extension of long, filamentous processes. We also noted that the control cells contained numerous clear droplets, presumably lipid, which were relatively uniform in size and mostly perinuclear in distribution, whereas the LZM-treated cells contained fewer droplets which were more irregular and scattered throughout the cytoplasm.

Although some of the cytological changes associated with LZM treatment of these cells might suggest cell damage and degeneration as a consequence of a non-specific toxic effect of LZM, the ^3H -thymidine incorporation data indicate that the net synthesis of DNA in these cultures was not markedly reduced by LZM.

Electron Microscopy

To document further the effects of LZM on the surfaces and organelles of parenchymal (hepatic) cells, scanning (SEM) and transmission electron microscopic (TEM) studies of human cell cultures were carried out (in collaboration with Professor Marcel Bessis and Dr Yves Le Charpentier at the

coverslip cultures were prepared and processed for transmission electron microscopy (Philips EM 200).

Twenty hours after plating in the presence of human LZM ($200\text{ }\mu\text{g ml}^{-1}$), both control and LZM-treated cells were firmly attached to the glass surface, and cell numbers were approximately the same (Fig. 5). Control cells (Figs 5A and C) were relatively more compact and spherical than the treated cells, and their cytoplasmic borders were sharply demarcated with only a few short, filamentous projections. In contrast, cells grown in the presence of LZM (Figs 5B and D) were markedly flattened and spread out on the glass surface, and their cytoplasmic borders had numerous long, filamentous projections. Also, the surfaces of the control cells appeared relatively smooth although a fine granularity was revealed by

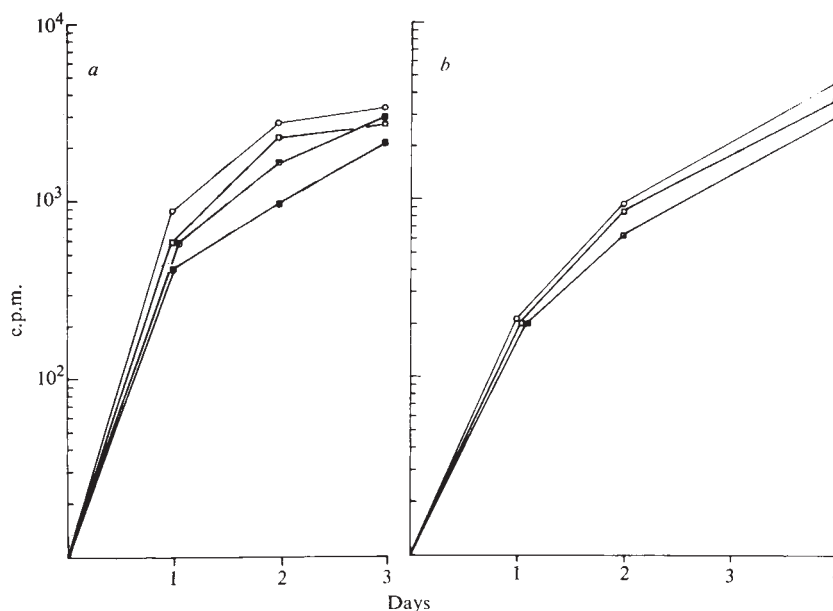


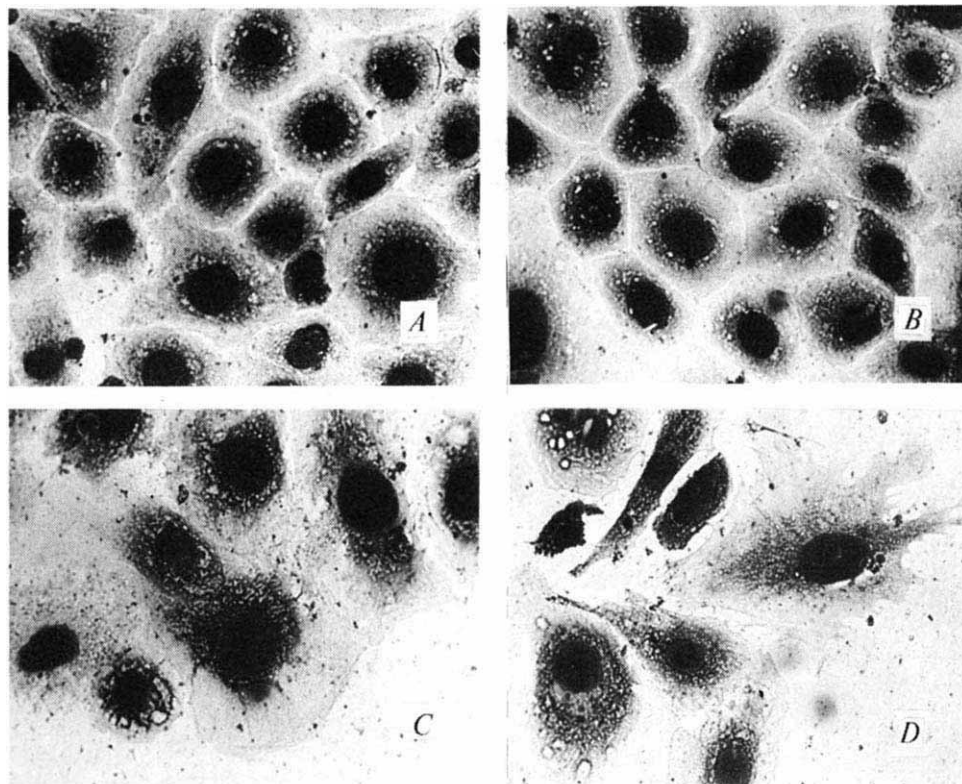
Fig. 3 Effects of increasing concentrations of rat LZM (200 to $800\text{ }\mu\text{g ml}^{-1}$) on the uptake of ^3H -thymidine by (a) normal (3T3) and (b) transformed (SV101-3T3) mouse embryo fibroblasts. Cells were grown as monolayers on $11 \times 22\text{ mm}$ hanging glass coverslips, according to the method described by Rytömaa⁵. The coverslips were initially placed along the down side of sterile plastic culture tubes ($16 \times 125\text{ mm}$) in the horizontal position. The volume (0.5 ml) between the lower surface of the coverslip and the test tube wall was then filled with a suspension of cells (10^3 to 10^4 per ml) in tissue culture medium containing ^3H -thymidine ($1\text{ }\mu\text{Ci ml}^{-1}$). After filling each tube, it was quickly rotated 180° along its horizontal axis bringing the coverslips to the top side, thus permitting the cells to settle on its surface. In this position the tubes were closed with loose fitting plastic caps and placed in the incubator. In a separate experiment, it was determined that the majority of 3T3 and SV-3T3 cells were adherent to the coverslip surface within 30 min. In all experiments, sufficient numbers of cultures were set up initially to permit collection of three or four cultures after 1, 2, 3 and 4 d of incubation. At these intervals, the coverslips were removed from the culture tubes, air dried, fixed in 95% ethanol and washed with distilled water. They were then placed in scintillation vials containing 10 ml of 'Aquasol' (New England Nuclear Corp) and counted in a Packard Tri-Carb liquid scintillation spectrometer. In previous studies⁵, it was established that the channel ratios and energy spectra of ^3H -thymidine labelled bone marrow cells attached to coverslips were not significantly different from those of cells suspended directly in the scintillation fluid or from ^3H -thymidine in solution. The initial cell concentration was 10^4 per ml for the 3T3 cells and 4×10^3 per ml for the SV-3T3 cells. Each point represents the mean of three to four cultures. $\circ$, Control; $\square$, $200\text{ }\mu\text{g ml}^{-1}$ rat LZM; $\square$, $400\text{ }\mu\text{g ml}^{-1}$ rat LZM; $\blacksquare$, $800\text{ }\mu\text{g ml}^{-1}$ rat LZM.

Institute of Cellular Pathology, Paris (Bicêtre)). The cell line used was originally derived from a liver biopsy of a 4-yr-old girl with haemolytic anaemia and microspherocytosis. The tissue culture was started in January 1972 and showed restricted growth for 2 months (four passages) at which time it apparently transformed spontaneously and proliferated more rapidly. At the time of our study (August 1972), the cells had been passaged fifty times. They were grown on glass coverslips in Leighton tubes using Eagle's medium supplemented with 20% foetal calf serum, glutamine, vitamins, heparin and antibiotics. After dehydration, the coverslips were air dried ('critical point' method) and the cells coated with a conducting layer of vacuum evaporated gold. The coverslips were attached directly to the specimen holder of the microscope (Cambridge-Stereoscan, operated at 30 kV). Pictures were taken at $\times 500$ – $6,000$ at about 40° to the monolayer surface. Parallel

higher magnification (Fig. 5C), whereas the surfaces of the LZM-treated cells (Fig. 5D) had patches of verrucous nodularity plus scattered, smooth-surfaced nodules. Cultures of these human liver cells with rat LZM ($200\text{ }\mu\text{g ml}^{-1}$) showed similar effects although less marked.

For the TEM studies (Fig. 6), cells were transferred directly from the glass surfaces and embedded for sectioning. Consistent with the SEM observations, the control cells displayed relatively smooth cytoplasmic margins whereas the cytoplasmic margins of the LZM-treated cells showed numerous villous projections. Fig. 6 shows further that the control cells contained numerous large, fat droplets principally clustered around the nuclei and probably responsible for the fine surface granularity seen by SEM. These droplets were virtually absent from the LZM-treated cells. Furthermore, in the control cells the mitochondria and ribosomes were irregular and poorly-

Fig. 4 Cytology of spontaneously transformed rat liver (RLB) cell cultures grown in control conditions and in the presence of rat LZM ($400 \mu\text{g ml}^{-1}$) for 2 and 4 d, respectively. After counting for ^3H -thymidine, the coverslips were washed in 95% ethanol to remove the scintillation fluid and then stained with Wright Giemsa stain for cytological examination. *A*, Control, 2 d; *B*, control, 4 d; *C*, rat LZM, 2 d; *D*, rat LZM, 4 d.



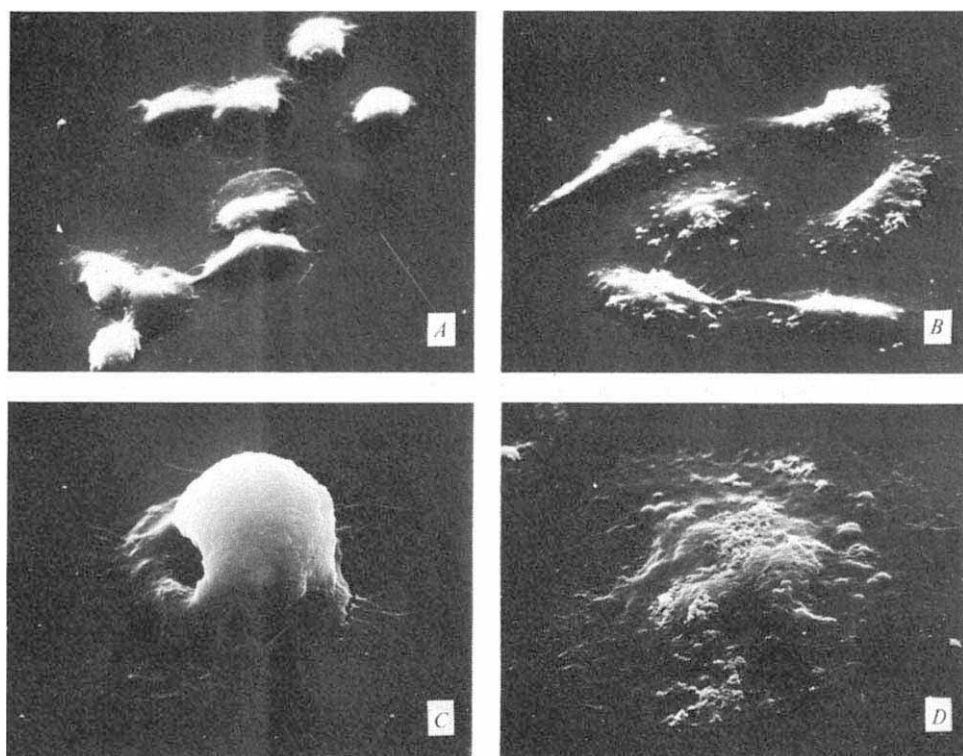
defined, whereas in the cells treated with LZM the mitochondrial membranes and cristae were more regular and sharply defined and the ribosomes more numerous and clustered in polysomes. Thus, with respect to certain features of internal structure, the LZM-treated cells appeared more normal than the untreated control cells. It also appeared that the outer ("plasma") membranes of the LZM-treated cells were thinner and more sharply defined than those of the

control cells, consistent with a reduction in the amount of surface polysaccharide ("glycocalyx").

Implications

Our results indicate that LZM has significant effects on various mammalian cells *in vitro*, apparently not due to non-specific toxicity. The changes in cell configurations from globular to flattened contours and the development of fila-

Fig. 5 Scanning electron micrographs of spontaneously transformed human liver cell cultures after 20 h *in vitro*. *A* and *C*, Control; *B* and *D*, human LZM ($200 \mu\text{g ml}^{-1}$). *A* and *B*, $\times 210$; *C* and *D*, $\times 1,092$.



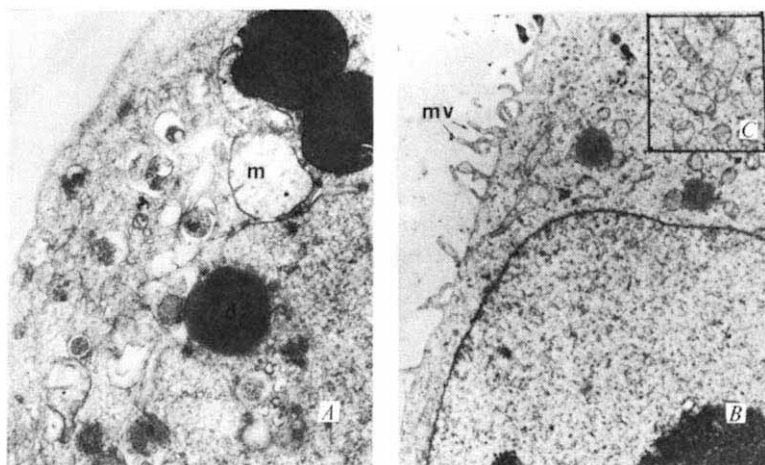


Fig. 6 Transmission electron micrographs of spontaneously transformed human liver cell cultures after 20 h *in vitro*. *A*, Control; *B*, human LZM (200 μ g ml⁻¹); *C*, detail of (*B*) upper right. *A*, $\times 27,675$; *B*, $\times 6,750$; *C*, $\times 27,675$. *M*, Mitochondrion; *d*, lipid droplet; *mv*, microvilli; *r*, ribosomes.

mentous cytoplasmic projections seen by electron microscopy imply a significant increase in total surface area, presumably accomplished either by extension ("stretching") of existing membrane or synthesis of new membrane, or both. The former could be due to the rearrangement of a lattice structure secondary to an effect of LZM on membrane polysaccharides or a non-specific polycation effect on the surface charge interactions with supporting surfaces⁷⁻¹¹. Even if these changes are due to polycation effects, they may be significant when LZM is released from macrophages onto the surface of target cells. Alternatively or additionally, if LZM causes these cells to produce more membrane, the numerous mitochondria and polyribosomes and the reduced number of lipid droplets in treated cells may be related to this synthesis.

There is considerable, although mostly circumstantial, evidence that macrophages and histiocytes in tumours of man¹²⁻¹⁷ and experimental animals^{15,17} are important in host defences against neoplasia^{15,16,18,19}. In addition, the antitumour effects of BCG vaccine and other adjuvants may be related to their ability to stimulate or activate macrophages²⁰⁻²⁶. The mechanisms and mediators involved are unknown, but LZM is elaborated by and released from macrophages^{27,28}, and increased production and/or release of LZM by macrophages has been demonstrated after BCG vaccination²⁸⁻³⁰. This information in conjunction with our findings suggests that LZM is a mediator of antitumour functions of macrophages. We also postulate that the cellular effects of LZM are due to a combination of its cationic properties, facilitating interaction with the negatively charged cell surfaces^{7,8} and an enzymatic effect on (a) membrane polysaccharide(s). With respect to the first property, the polyanion heparin which strongly inhibits LZM activity against bacterial substrates^{31,32} also inhibits the immune cell-mediated killing of mastocytoma cells³³.

We obtained our *in vitro* effects using homologous mammalian LZMs which can be isolated in gramme quantities from the urine of patients and rats with monocytic leukaemia^{3,33}, and which are apparently identical to their normal counterparts³. Further, the concentrations of LZM we used were probably within the range achieved *in vivo* at sites where macrophages interact with target cells.

Finally, we found relatively little *in vitro* toxicity of LZM, and some preliminary animal studies (unpublished results of E. F. O. *et al.*) indicate that relatively large amounts of homologous LZM (up to 5 mg g⁻¹ body weight, administered *i.v.* or *i.p.*, daily for several weeks) are well tolerated by mice and rats. Thus, if the biological usefulness of LZM is established, its pharmacological use is probably feasible.

We thank Professor Marcel Bessis and Dr Yves Le Charpentier for the electron microscopy studies and Miss Judith Weisfuse and Mrs Carmen Colon for technical assistance. These studies were supported by a grant from the National

Cancer Institute. E. F. O. is an American Cancer Society Professor of Medicine.

Received January 2, 1973.

- ¹ Fleming, A., *Proc. Roy. Soc., B*, **93**, 306 (1922).
- ² Imoto, T., Johnson, L. N., North, A. C. T., Phillips, D. C., and Rupley, J. A., in *The Enzymes*, third edit. (edit. by Boyer, P. D.) (Academic Press, New York, 1972).
- ³ Osserman, E. F., and Lawlor, D. P., *J. Exp. Med.*, **124**, 921 (1966).
- ⁴ Rosenthal, D. S., and Moloney, W. C., *Proc. Soc. Exp. Biol. Med.*, **126**, 682 (1967).
- ⁵ Rytömaa, T., *In Vitro*, **4**, 47 (1969).
- ⁶ Borek, C., *Proc. US Nat. Acad. Sci.*, **69**, 956 (1972).
- ⁷ Katchalsky, A., *Biophys. J.*, **4**, 9 (1964).
- ⁸ Ambrose, E. J., in *The Biology of Cancer* (edit. by Ambrose, E. J., and Roe, F. J. C.), 65 (Van Nostrand, London, 1966).
- ⁹ Moroson, H., *Cancer Res.*, **31**, 373 (1971).
- ¹⁰ Weiss, L., and Zeigel, R., *J. Cell. Physiol.*, **77**, 179 (1971).
- ¹¹ Weiss, L., Zeigel, R., Jung, O. S., and Bross, I. D. J., *Exp. Cell Res.*, **70**, 57 (1972).
- ¹² Black, M. M., Kerpe, S., and Speer, F. D., *Amer. J. Pathol.*, **29**, 505 (1953).
- ¹³ Hamlin, I. M. E., *Brit. J. Cancer*, **22**, 383 (1968).
- ¹⁴ Silverberg, S. G., Chitale, A. R., Hind, A. D., Frazier, A. B., and Levitt, S. H., *Cancer*, **26**, 1177 (1970).
- ¹⁵ *Role du Système Réticulo-Endothélial dans l'Immunité Anti-Bactérienne et Anti-Tumorale* (edit. by Halpern, B. N.), No. 115 (Editions du Centre National de la Recherche Scientifique, Paris, 1953).
- ¹⁶ Thomas, L., in *Cellular and Humoral Aspects of the Hypersensitive States* (edit. by Lawrence, H. S.), 529 (Hoeber-Harper, New York, 1959).
- ¹⁷ Aoki, T., Teller, M. N., and Robitaille, M.-L., *J. Nat. Cancer Inst.*, **34**, 255 (1965).
- ¹⁸ Old, L. J., Benacerraf, B., Clarke, D. A., Carswell, E. A., and Stockart, E., *Cancer Res.*, **21**, 1281 (1961).
- ¹⁹ Old, L. J., Clarke, D. A., Benacerraf, B., and Goldsmith, M., *Ann. NY Acad. Sci.*, **88**, 264 (1960).
- ²⁰ Old, L. J., Clarke, D. A., and Benacerraf, B., *Nature*, **184**, 291 (1959).
- ²¹ Weiss, D. W., Bonhag, R. S., and Leslie, P., *J. Exp. Med.*, **124**, 1039 (1966).
- ²² Mathé, G., Schwarzenberg, L., Amiel, J. L., *et al.*, *Cancer Res.*, **27**, 2542 (1967).
- ²³ Mathé, G., Pouillart, P., and Lapeyraque, F., *Brit. J. Cancer*, **23**, 814 (1969).
- ²⁴ Mathé, G., Amiel, J. L., Schwarzenberg, L., *et al.*, *Lancet*, **i**, 697 (1969).
- ²⁵ Bluming, A. Z., Vogel, C. L., Ziegler, J. L., Mody, N., and Kamy, G., *Ann. Intern. Med.*, **76**, 405 (1972).
- ²⁶ Zbar, B., and Tanaka, T., *Science*, **172**, 271 (1971).
- ²⁷ Myrvik, Q. N., Leake, E. S., and Fariso, B., *J. Immunol.*, **86**, 133 (1961).
- ²⁸ Cohn, Z. A., and Wiener, R., *J. Exp. Med.*, **118**, 991 (1963).
- ²⁹ Myrvik, Q. N., Leake, E. S., and Oshima, S., *J. Immunol.*, **89**, 745 (1962).
- ³⁰ Carson, M. E., and Dannenberg, jun., A. M., *J. Immunol.*, **94**, 99 (1965).
- ³¹ Nihoul, E., Massart, L., and Van Heel, G., *Arch. Intern. Pharmacodynamie*, **88**, 123 (1951).
- ³² Kerby, G. P., and Eadie, G. S., *Proc. Soc. Exp. Biol. Med.*, **83**, 111 (1953).
- ³³ Martz, E., and Benacerraf, B., *Fed. Proc.*, **31**, 786 (1972).

LETTERS TO NATURE

PHYSICAL SCIENCES

Redshift of OQ172

WE report the discovery of a second QSO with a redshift greater than 3. Spectra taken at the 120-inch at Lick Observatory¹ give $z=3.53$ for OQ172. Carswell and Strittmatter² have found the redshift of OH471 to be $z=3.40$. Our attention was drawn to the object by the work of Gent *et al.*³ on Molonglo radio sources. Accurate radio coordinates for OQ172 were communicated to us ahead of publication by C. Hazard and H. Gent. They are:

$$\alpha (1950) 14 \text{ h } 42 \text{ min } 50.48 \text{ s} \\ \delta (1950) 10^\circ 11' 12.4''$$

A finding chart for OQ172 is given by Véron⁴, who notes that it is a "blue stellar object". From our inspection of the Palomar Sky Survey plates we agree with Véron's assessment of the colour of OQ172, although Gent *et al.*³ list it as stellar, that is an object with neutral colours. Radio fluxes for OQ172 are available from ref. 5, where the spectral index is given as -0.09 between 178 and 1,400 MHz. The tabulated fluxes at 1,400, 2,695 and 5,009 MHz are 2.42, 1.96 and 1.22 f.u., respectively⁵. The radio spectrum is therefore very flat and extends to high frequencies.

Figure 1 shows a spectrum of OQ172 obtained with the Cassegrain ITS system⁶ at Lick Observatory. This spectrum is a composite formed by adding data from spectra obtained on different nights. Consequently the signal-to-noise ratio in the spectrum varies as a function of wavelength. It is particularly poor in the $\lambda 6000$ – $\lambda 6500$ region where we have only one spectrum, to the red of $\lambda 7200$ Å where OH emission features increase the radiation from the night sky, and shortward of 3500 Å where the atmospheric extinction becomes troublesome. In the spectral region between 4000 Å and 6000 Å the signal-to-noise ratio is good; most of the strong features present here are real and can be seen on the several individual spectra. The two strong emission features near $\lambda 5540$ and $\lambda 7015$ have been identified with Ly- α $\lambda 1216$ and CIV $\lambda 1549$. The measured wavelength of the emission peaks corresponds to a redshift of 3.56 for Ly- α and 3.53 for CIV. The very strong absorption in the blue wing of the feature identified as Ly- α has probably shifted the measured wavelength to the red of the true peak. For this reason we do not regard the slight difference in the measured redshifts of the lines as being significant. We found no other acceptable fit to the observed ratio of wavelengths that could not be rejected because of the absence of other strong lines in observed spectral regions. The presence of many strong absorption lines would also suggest a large redshift, say $z \lesssim 2$. We therefore conclude that the emission line redshift is near 3.53. The position of several features assuming a redshift of 3.53 have been marked on Fig. 1.

It is common for large redshift QSOs to have absorption features; OQ172 seems to have one of the richest absorption spectra known. The heavy absorption shortward of Ly- α

increases the difficulty of identifying emission features in that spectral region. There is some indication of emission at the expected position of Ly- β and OVI but spectra with higher resolution will be needed to reduce the line blending and establish the continuum level before any such identification can be regarded as secure. In any case, there is no doubt that the continuum radiation remains strong far to the violet of the position of the Lyman edge in the emission line redshift system, a surprising result for an object with such strong absorption features. The strongest lines near the Ly- α emission peak have the correct separation to be identified with the CIV $\lambda 1549$ doublet at $z=2.56$, raising the possibility that the absorption line systems have a much lower redshift than the emission line system. The Lyman continuum absorption associated with these features would then begin at the extreme violet end of our data where the signal-to-noise ratio is poor. It is also possible, in a gas with little turbulent motion, to have a substantially higher optical depth in Ly- α than in the Lyman continuum. One could therefore have strong, narrow, Ly- α absorption features without having appreciable absorption in the Lyman continuum. A programme to obtain higher resolution spectra of this object is now under way and should help resolve some of these questions.

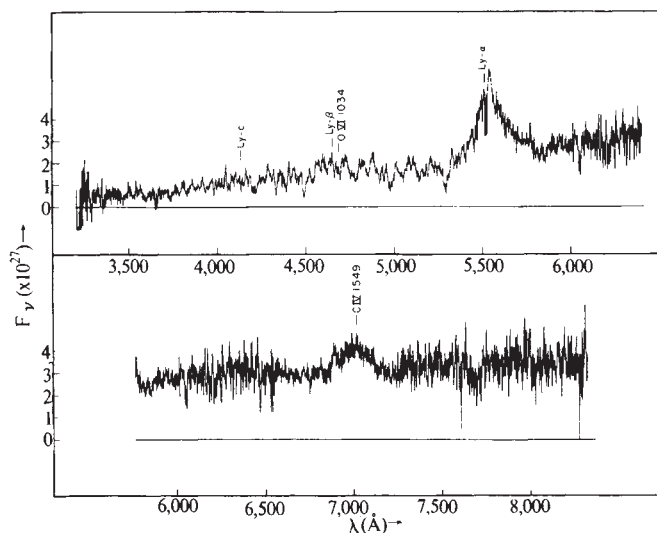


Fig. 1 Image tube scanner spectrum of OQ172. Units of F_v are $\text{erg cm}^{-2} \text{s}^{-1} \text{Hz}^{-1}$.

OQ172 is very bright. It was estimated by Véron⁴ to be magnitude 17.5. Our calibrated spectra, which should be accurate to $\pm 20\%$, indicate that the V magnitude of the continuum is about 17.9. The strong continuum level, extending well into the ultraviolet, is in agreement with the blue classification given to OQ172 by Véron⁴. Although it is bright at short radio wavelengths it is too faint to appear in the 4C catalogue, otherwise it satisfies all the normal criteria for classification as a QSS. Other, similar objects presumably

exist and could be discovered by the proper survey techniques without requiring highly accurate radio positions.

This research has been supported, in part, with grants from the National Science Foundation, the National Aeronautics and Space Administration and NATO.

E. J. WAMPLER
L. B. ROBINSON
J. A. BALDWIN

Lick Observatory,
Board of Studies in Astronomy and
Astrophysics,
University of California,
Santa Cruz

E. M. BURBIDGE *

Royal Greenwich Observatory,
Herstmonceux Castle

Received May 14, 1973.

* On leave of absence from the Department of Physics, University of California, San Diego.

¹ Fitch, L. T., Dixon, R. S., and Kraus, J. D., *Astron. J.*, **74**, 612 (1969).

² Carswell, R. F., and Strittmatter, P. A., *Nature*, **242**, 394 (1973).

³ Gent, H., Crowther, J. H., Adgie, R. L., Hoskins, D. G., Murdoch, H. S., Hazard, C., and Jauncey, D. L., *Nature*, **241**, 261 (1973).

⁴ Véron, M. P., *Astron. Astrophys.*, **11**, 1 (1971).

⁵ Witzel, A., Véron, P., and Véron, M. P., *Astron. Astrophys.*, **11**, 171 (1971).

⁶ Robinson, L. B., and Wampler, E. J., *Publ. Astron. Soc. Pacific*, **84**, 161 (1972).

Intensity and Extent of Rain on Earth-Space Paths

EXISTING broadband satellite communications systems operate at carrier frequencies such as 4 and 6 GHz where bandwidths of about 0.5 GHz are available. At frequencies of 15 to 40 GHz, however, bandwidths of the order 3 GHz are possible but propagation through rain presents difficulties because there is significant absorption and scattering at the higher frequencies¹.

Here we examine attenuation data obtained simultaneously at 16 and 30 GHz using a Sun tracker² in New Jersey. The Sun

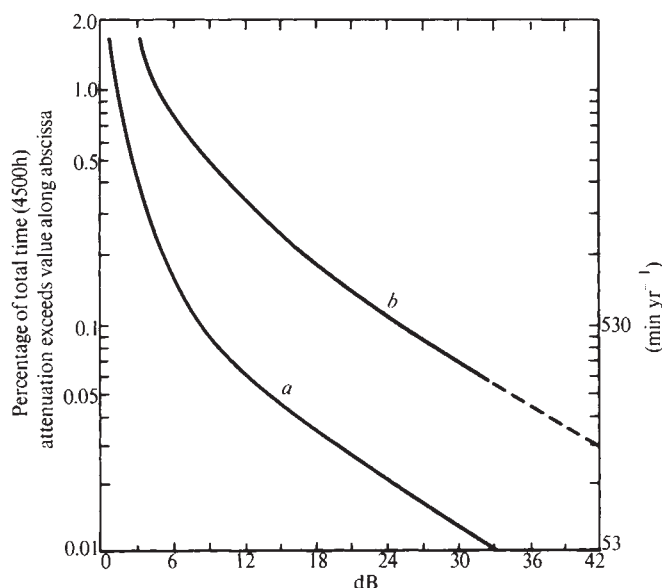


Fig. 1 Distribution of attenuation by rain measured simultaneously (in daytime) at 16 (a) and 30 (b) GHz by the Crawford Hill Sun tracker during 1968.

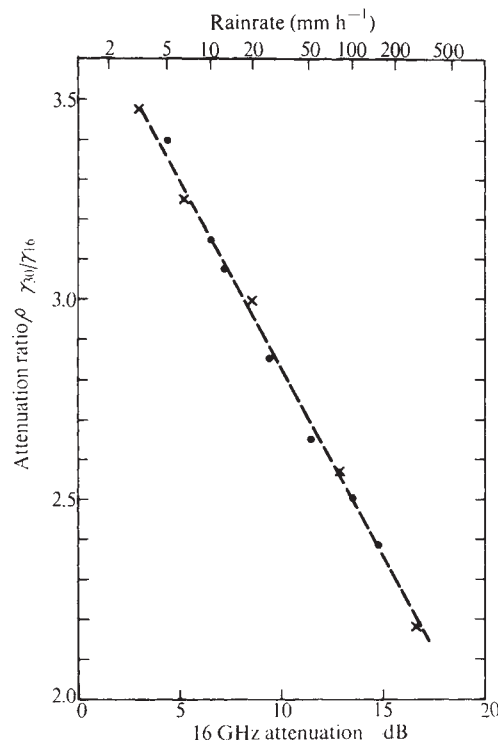


Fig. 2 Measured ratio of attenuations, obtained from the distributions of Fig. 1 against attenuation, and calculated ratio of attenuations against rain rate. $\times$, ρ_m , measured; $\bullet$, ρ_c , calculated.

is a source of quite constant power at these frequencies. The instrument is capable of measuring rain attenuation up to 35 dB at both frequencies with a time constant of 2 s. Operation is of course limited to the daytime, but the data base thus obtained amounts to about 4,000 h yr⁻¹.

Comparison of the statistical behaviour of signals at two or more frequencies propagated over a given path has been useful in the past for determining the transmission properties of the troposphere at both radio²⁻⁵ and optical⁶ frequencies. In the present case, the ratio of attenuation at 30 GHz to that at 16 GHz, obtained from cumulative distributions of attenuation, is used to estimate the intensity and extent of rain showers on an Earth-space path. To do this, it is necessary to specify a size distribution for the raindrops. We choose the classical (measured) Laws-Parsons distribution⁷ which represents adequately the drop sizes in most rains. A salient property of this size distribution is that the more intense the rain, the more abundant are drops of large diameter.

The cumulative probability distributions of attenuation at 16 and 30 GHz for the days of 1968 are shown in Fig. 1. Because the radiation from the Sun at both frequencies is received on the same antenna (beamwidths 0.9 and 0.5°), the attenuated signals resulting from rain showers passing through the Earth-space path are time-wise in step; attenuation maxima and minima coincide. Therefore the ratio, ρ_m , of measured attenuations at a given probability is significant; for example, at the 0.1% level, $\rho_m = \gamma_{30}/\gamma_{16} = 25.5/8.5 = 3.0$. When this procedure is carried out for a number of probability levels, one obtains the crosses plotted in Fig. 2 which show that the measured ratio, ρ_m , decreases from 3.5 to 2.2 as the 16 GHz attenuation increases from 3 to 16 dB.

We now assume that the rain rate is constant over a segment of the path and that the size distribution of the raindrops is, at least on the average, the Laws-Parsons distribution. The calculated ratio, ρ_c , for various rain rates can then be obtained from computations based on the Mie theory⁷. In Fig. 2 ρ_c is plotted against rain rate as solid dots; the calculated attenuation ratio decreases from 3.5 to 2.4 as the rain rate increases

from about 5 to 150 mm h⁻¹. This behaviour follows from the greater abundance of large drops at high rain rates.

Clearly, both plots are well represented by the dashed line in Fig. 2; thus a direct comparison can be made between them in the following sense. When the 16 GHz attenuation is 8 dB, the measured attenuation ratio is 3.0. But that ratio is represented by a rain rate of 15 mm h⁻¹; therefore, when the attenuation on the path is 8 dB, the rain rate on the path is, on the average, 15 mm h⁻¹. For an attenuation of 13.5 dB, $\rho=2.5$, the apparent rain rate is 100 mm h⁻¹, and so on.

The equivalence between attenuation and rain can be used to derive a cumulative distribution of apparent rain rate; thus from Figs 1 and 2, one obtains Fig. 3. The abscissa is labelled "apparent" rain rate, R_a , for two reasons: to ensure that it is not confused with path-average rain rate, and because, on an Earth-space path, it represents density of rain rather than an actual rate, the velocity of fall in many convective rains being unknown.

If this derived distribution is compared with the point rain rate as measured by a rain gauge located at some point near the antenna, it is found that the probability occurrence of the high rates is much greater on the path than at the point. This is not surprising because it is obvious that a rain shower, appearing randomly over an area, is much more likely to intersect a line than to encompass a point.

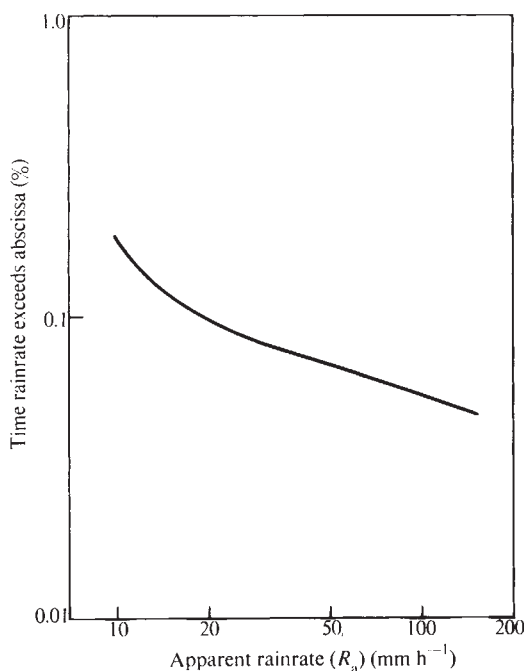


Fig. 3 Cumulative distribution for the apparent rain rate on the Earth-space path.

Up to this point, we have only been concerned with the ratio of attenuations. But the absolute value of attenuation is also known, as is the relationship between attenuation and path length, for a given uniform rain rate. If l is the extent of a rain of rate R_a on a path of arbitrary overall length, the attenuation is simply

$$\gamma_p = l\alpha(R_a)$$

where $\alpha(R_a)$ is the known attenuation coefficient⁷. γ_p is known from the measurements (Fig. 1) and $\alpha(R_a)$, for the same probability level, can be calculated using Fig. 3. Therefore the extent or path length, l , through the rain of apparent rate R_a can be obtained using the above relationship; it is shown in Fig. 4.

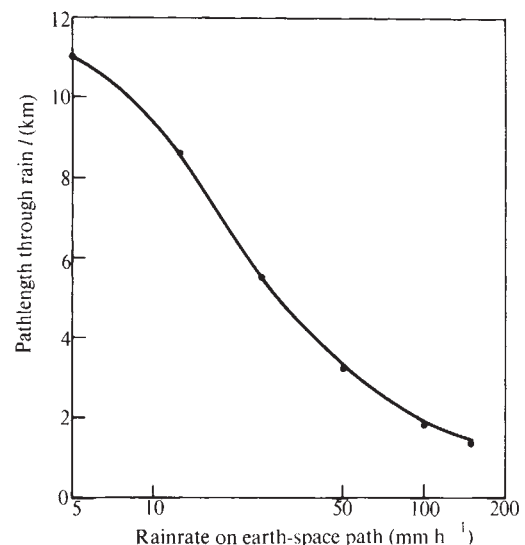


Fig. 4 Extent of rain on the Earth-space path against rain rate.

Figure 4 shows that at low rain rates the extent of the "cell" on the path is large, 10 km or more. On the other hand, when rain rate exceeds 100 mm h⁻¹, the extent of the cell is less than 2 km. Thus we end up with the common-sense result that the less intense rains on the average cover large areas whereas heavy showers are quite limited spatially. One can also plot the extent of the rain as a function of attenuation at 16 or 30 GHz and the behaviour is similar to that of Fig. 4—high attenuations are caused by relatively small cells. (This fact is basic to the technique of path diversity in millimetre-wave satellite communication systems.)

What is the practical significance of the method outlined above? A point on the plot in Fig. 4 gives the path length through a shower of given rain rate, and each point is associated with a certain probability. Therefore one can use those data to compute the attenuation statistics at any desired frequency where the Laws-Parsons drop-size distribution is applicable, resulting in a cumulative attenuation distribution for that frequency. Evans⁸ has applied the method in satellite-system calculations.

There are at least two criticisms to be made of this technique. First, the basic data (Fig. 1) were taken using a Sun tracker which encounters paths of various overall length through the atmosphere during a day. Dual-frequency data taken on a path at a fixed elevation angle, such as to a synchronous satellite, would be more satisfactory for the analysis. Second, the Laws-Parsons drop-size distribution has been assumed throughout. There is evidence⁵, however, that that distribution is satisfactory on terrestrial paths at the frequencies of interest. On the other hand, comparison of the plots in Fig. 2 promotes some confidence in its applicability in the present case. It is not known how sensitive the method is to a given drop-size distribution.

The technique amounts to remote probing, in the statistical sense, of the gross features of the rain medium; it is applicable to terrestrial as well as Earth-space paths.

D. C. HOGG

Bell Telephone Laboratories,
Holmdel, NJ 07733

Received August 10; revised November 17, 1972.

- ¹ Hogg, D. C., *Science*, **159**, 39 (1968).
- ² Wilson, R. W., *Bell System Tech. J.*, **48**, 1383 (1969).
- ³ Crawford, A. B., *et al.*, *Bell System Tech. J.*, **38**, 1067 (1959).
- ⁴ Wrixon, G. T., *Bell System Tech. J.*, **50**, 103 (1971).
- ⁵ Semplak, R. A., *Bell System Tech. J.*, **50**, 2599 (1971).
- ⁶ Chu, T. S., and Hogg, D. C., *Bell System Tech. J.*, **47**, 723 (1968).
- ⁷ Setzer, D. E., *Bell System Tech. J.*, **49**, 1873 (1970).
- ⁸ Evans, H. W., *Proc. Intern. Comm. Conf.* (Montreal, 1971).

Stress Diffusion from Plate Boundaries

ACCORDING to global tectonics, the outer relatively strong layer of the solid Earth (lithosphere) is divided into seven major and several minor discrete plates separated from each other by the Earth's mobile belts. The mechanism of global tectonics is believed to depend partly or wholly on the application and release of strain energy at mobile belts which lie along plate margins. The isostatic response of the lithosphere to application or removal of a wide surface load such as an icecap is delayed by the viscous response of the underlying asthenosphere to elastic bending of the lithosphere¹. By analogy, the viscous drag of the asthenosphere should also delay the response of the lithospheric plates to relief or application of horizontal boundary stresses on a plate edge, as indicated by an earlier calculation made by Elsasser². We have investigated this using a simple plane two-dimensional model of an elastic plate underlain by a viscous layer. We confirm that the viscous shearing stress of the asthenosphere on the base of the lithosphere is a significant factor in causing stress to diffuse through the lithosphere. We also predict the possible occurrence of a new type of strain wave in the lithosphere, with some resemblance to Voigt waves^{3,4}.

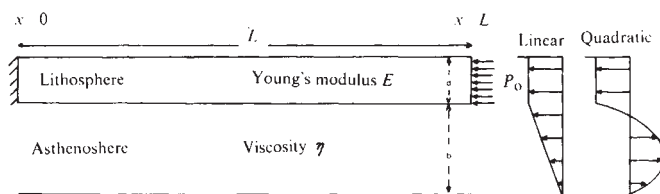


Fig. 1 Two-dimensional model of the lithosphere overlying the asthenosphere, with linear and quadratic velocity-depth distributions shown at the right side.

Our theoretical model consists of a horizontal two-dimensional elastic plate of length L , thickness d and Young's modulus E representing a lithospheric plate, underlain by a viscous layer of thickness b and Newtonian viscosity η representing the asthenosphere (Fig. 1). The viscous layer overlies a rigid and stationary half-space. The Earth's curvature is neglected and plane stress is assumed. We study the variation of horizontal pressure P and horizontal displacement $u(x,t)$ in the direction of the x axis as a function of distance x and time t . Inertial forces were included in some of our calculations but their effect was negligible for parameters compatible with the Earth.

By considering the horizontal forces acting on a small length of the elastic plate and neglecting inertial forces, one obtains an equation of motion for the displacement $u(x,t)$ of the part of the plate at point x at time t . This is²

$$\frac{\partial^2 u}{\partial x^2} = \sigma \frac{\partial u}{\partial t} \quad (1)$$

where $\sigma = \eta/bdE$ for an assumed linear velocity-depth distribution across the viscous layer or $\sigma = 2\eta(3d+2b)/b^2dE$ for a quadratic distribution such that no net transport occurs across a section perpendicular to the x axis. The horizontal variation of $P(x,t)$ within the elastic plate implies the existence of a bending moment which will cause small vertical movements, but we ignore this effect in the present exploratory investigation.

Equation (1) also applies to the propagation of a horizontal shear stress applied at the edge of the plate provided that E is replaced by the rigidity modulus μ . Equation (1) has identically the same form as the unidimensional equations of heat conduction or diffusion. The equation can be solved for given initial and boundary conditions by standard methods⁵.

A simple investigation which gives much insight is to determine the response of an elastic plate of semi-infinite length to a sinusoidal boundary pressure $P = P_0 \sin \omega t$ of period $T = 2\pi/\omega$ applied at the end $x=0$. The appropriate solution of equation (1) is

$$u(x,t) = -\frac{P_0}{\sqrt{2kE}} e^{-kx} \cos\left(\omega t - kx + \frac{\pi}{4}\right) \quad (2a)$$

and

$$P(x,t) = P_0 e^{-kx} \sin(\omega t - kx) \quad (2b)$$

where $k = \sqrt{\frac{1}{2}\omega\sigma}$. This represents a train of stress (or strain) waves of period T whose maxima and minima travel along the x -direction with velocity $v = \omega/k$, with amplitude falling off exponentially with distance. Defining the penetration X as the distance to the point where the amplitude falls off to e^{-1} of its value at $x=0$, we have for the linear velocity in the viscous layer

$$X = \sqrt{\frac{ETbd}{\pi\eta}} \text{ and } v = 2\sqrt{\frac{\pi Ebd}{\eta T}} \quad (3)$$

We calculated representative values to indicate the order of magnitude of the penetration and velocity assuming $d=80$ km, $b=250$ km, $\eta=2 \times 10^{21}$ P, and $E=10^{12}$ dyne cm⁻² (Table 1).

Table 1 Representative Values of Model Parameters

T (yr)	X (km)	v (km yr ⁻¹)
1	10	62.8
10 ²	100	6.28
10 ⁴	1,000	0.628
10 ⁶	10,000	0.0628

For the quadratic velocity-depth distribution in the viscous layer, the values of X and v in the table should be divided by 2.43.

One implication of this result is that a sudden change of the boundary stress at the edge of a plate (such as might result from an earthquake) would not be expected to penetrate into the interior of the plate except over a long period of time, except insofar as the asthenosphere can respond elastically⁶.

Another implication is that one might expect to observe stress waves in the vicinity of plate boundaries where boundary stresses vary with time, travelling away from the boundary with a velocity inversely proportional to the square root of period. The penetration of waves of period of up to 100 yr would be restricted to a few hundred kilometres from the plate margin. An example of such stress waves has been presented by Kasahara⁷, based on tiltmeter records at two stations in Japan about 20 km apart. Crustal strains of dominant period about one to four years appear to migrate from east to west (away from the surface position of the Benioff zone) at a velocity of about 20 km yr⁻¹. The velocity is in good agreement with the predictions of the table above, although the origin of the stress variation appears to be closer than the Japan trench which is 200 km away.

If our model is correct, search for secular strain variations should be made in the vicinity of plate boundaries but not in the interior regions of plates.

We now investigate the response of the elastic plate of finite length L to the application of a constant pressure at one end representing an ocean ridge, and to the release and subsequent rebuildup of strain at the other end, representing a compression plate boundary such as an island arc-trench system. Although the models must be treated as severe oversimplifications, particularly for the release of strain, they do give significant insight into the relevant processes.

For the first problem, the end of the plate at $x=0$ is regarded as fixed and represents a compression boundary. A constant

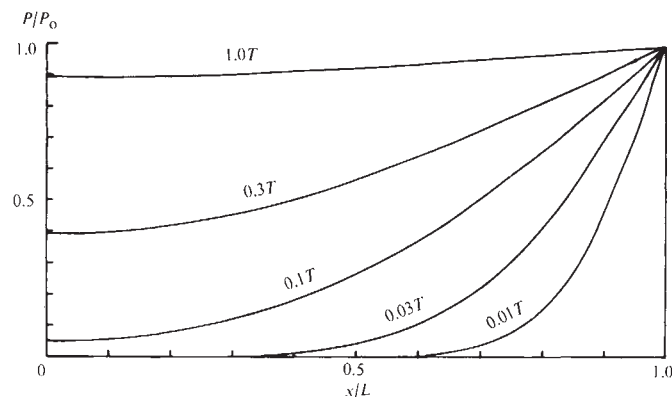


Fig. 2 The horizontal pressure distribution in the lithosphere plotted for a series of values of time after application of a pressure P_0 at the end $x=L$ at time $t=0$, where time $T=\sigma L^2$. If $L=10,000$ km, $d=80$ km, $b=250$ km, $\eta=2 \times 10^{21}$ P and $E=10^{12}$ dyne cm^{-2} , then $T=316,900$ yr for the linear velocity-depth distribution and $T=1.87 \times 10^6$ yr for the quadratic distribution.

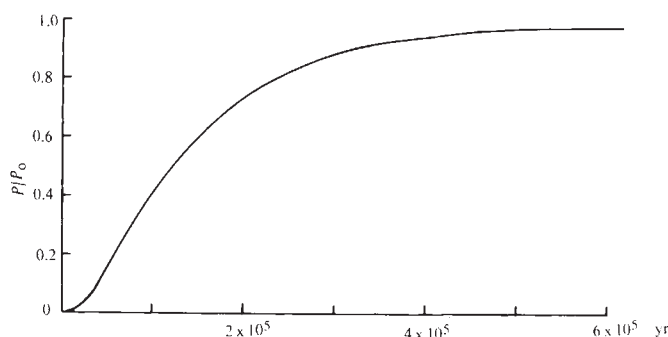


Fig. 3 Graph showing the buildup of horizontal pressure P at $x=0$ as a function of time after application of pressure P_0 at $x=L$ for the linear velocity-depth distribution assuming the values of parameters given in legend of Fig. 2.

pressure P_0 is instantaneously applied at the end $x=L$ representing the ocean ridge at time $t=0$. Equation (1) must then be solved for the initial condition $u=0$ at $t=0$ ($0 \leq x < L$), and for the boundary conditions $u=0$ at $x=0$ ($t > 0$) and $\left(\frac{\partial u}{\partial x}\right)_{x=L} = -P_0/E$, $t > 0$.

The resulting pressure distribution along the plate has been plotted in a non-dimensional form in Fig. 2 for values of time t shown on each wave, where $T=\sigma L^2$. Figure 3 shows the buildup of pressure at the fixed end A as a function of time for specific values of the dimensions and physical properties (it can be scaled readily for other values). These examples show that it takes about 10^5 to 10^6 yr for the pressure to approach the value P_0 throughout the plate if $L=10,000$ km, and of the order of 10^3 to 10^4 yr if $L=1,000$ km.

If the end A is interpreted as a compression boundary of a plate, then as pressure increases at A (Fig. 3) it will eventually reach a critical value P_c at time τ when resistance is overcome and the edge of the plate is displaced (for example down a Benioff zone). In our final model, we examine how the pressure will rebuild at A after such stress relaxation. We simulate stress relaxation at and near A by assuming an instantaneous displacement $u_+ = -\frac{P_c s}{E} e^{-x/s}$ at time τ , where s is a parameter

which controls the fall-off of the stress changes with distance from the origin. A computed plot of the buildup of pressure at A after stress relaxation, assuming $P_c=526$ bar and $s=3$ km, is shown in Fig. 4. This shows that the stress in the vicinity of the boundary builds up again in a period of 1 to 10^6 yr. When

the pressure again reaches the critical value the relaxation will be repeated, and because the timescale in Fig. 3 is so much larger than in Fig. 4 one would expect the initial conditions after the second relaxation to be almost identical to those after the first. Thus the pressure at A would be expected to cycle as in Fig. 5.

In the example (Fig. 4), the instantaneous displacement at $x=0$ for $s=3$ km is 150 cm and the release of strain energy is 0.6×10^{18} erg cm^{-1} length perpendicular to the model. Our model of stress release is a serious oversimplification, and in practice the stress drop would only be partial and would vary with depth. One would expect a smaller instantaneous displacement and release of strain energy occurring more frequently. In spite of our simplification of the problem, it is easy to see that plates can move forward at velocities of the order of 6 cm yr^{-1} by this mechanism.

On our model the plates are driven by horizontal pressure applied at or near ocean ridges. We have shown that short period cyclical variations of pressure at the ridges do not penetrate into the interior of a plate and that persistent changes in pressure take of the order of a million years to penetrate across a large plate. At the compression boundary, instantaneous release of strain energy probably only affects a few tens of kilometres at most from the boundary and the stresses then build up towards their original value over a period of the order of 1 to 100 yr. Thus the model predicts that release of strain energy at a compression boundary will occur periodically and this part of the plate will move forward in a series of jerks. The principal interior part of the plate acts as a reservoir for strain energy, isolating the opposite boundaries from each other, and moving forward at a uniform velocity as long as the applied pressure at the ridge averaged over a suitably long period remains constant.

Another possibility is that lithospheric plates are driven by

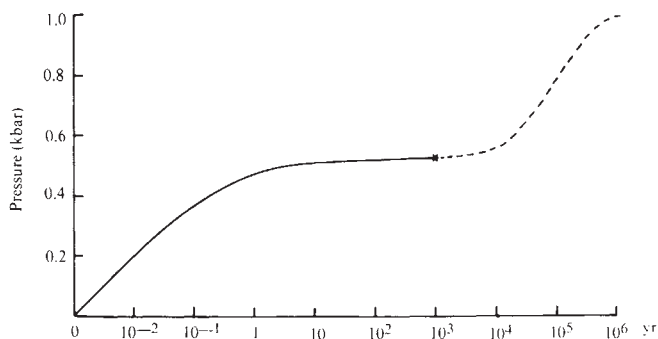


Fig. 4 The increase in horizontal pressure P at $x=0$ after strain release as described in text. Failure would be expected to occur again at X.

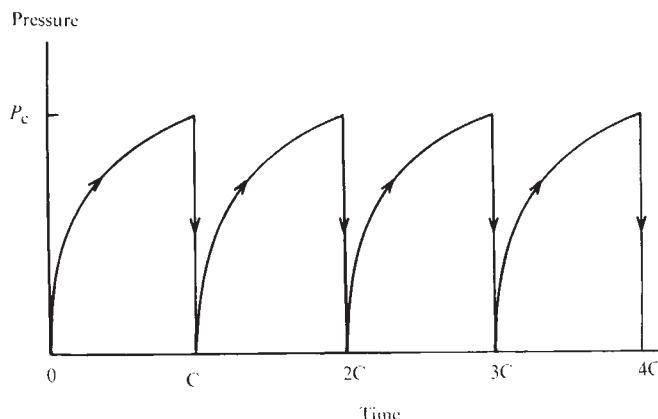


Fig. 5 Diagram to illustrate qualitatively the periodic release of strain at a compression boundary at time interval C , as predicted by our model.

drag of underlying convection currents. We have not studied this model of plate mechanism, but similar considerations would be expected to apply to the response of the plate to changes in the driving stresses and to release of strain energy at compression boundaries.

We have shown that stress suddenly applied or released at a plate boundary cannot instantaneously affect the whole plate but diffuses through it over a period of the order of a thousand to a million years depending on dimension. The crucial factor causing this slow stress migration is the viscosity of the asthenosphere. If the stress variation at a plate boundary is periodic, then a decaying train of stress waves will pass into the plate with velocity depending on period and physical properties of the order of 0.1 to 10 km yr⁻¹. Finally, if our model of stress diffusion in the lithosphere is substantiated by future observations, it may become, in association with the theory of creep wave propagation along transform faults⁸, an important factor in prediction of earthquakes and volcanic activity near compression boundaries.

We thank Dr G. A. L. Johnson for reviewing the manuscript. D. S. D. held a NERC research studentship.

M. H. P. BOTT
D. S. DEAN

Department of Geological Sciences,
University of Durham

Received February 6, 1973.

¹ Haskell, N. A., *Physics*, 6, 265 (1935).

² Elsasser, W. M., in *The Application of Modern Physics to the Earth and Planetary Interiors* (edit. by Runcorn, S. K.), 223 (Wiley-Interscience, 1969).

³ Collins, F., *Geophysics*, 25, 483 (1960).

⁴ Clark, G. B., and Rupert, G. B., *J. Geophys. Res.*, 71, 2047 (1966).

⁵ Carslaw, H. S., and Jaeger, J. C., *Conduction of Heat in Solids* (Oxford Univ. Press, 1959).

⁶ Press, F., *J. Geophys. Res.*, 70, 2395 (1965).

⁷ Kasahara, K., *Phil. Trans. Roy. Soc.* (in the press).

⁸ Savage, J. C., *J. Geophys. Res.*, 76, 1954 (1971).

Evidence for Buffering of Dissolved Silicon in Fresh Waters

THE widely used colorimetric method for determining dissolved silicon in natural waters measures only monomeric or low molecular weight polymers of silicic acid¹. But there seems little reason to believe that appreciable amounts of higher, and therefore unreactive, polymers exist in the sea or most terrestrial waters^{2,3}.

Livingstone⁴, in the most extensive compilation of river water analyses to date, obtains a worldwide mean dissolved silicon concentration of 13.1 mg SiO₂ l⁻¹. These data show that the concentration of silicon in rivers is remarkably constant compared with other major dissolved constituents (for example, the coefficient of variation for all tabulated silicon values is approximately one fifth that for total dissolved salts). This finding is reinforced by the discovery⁵ that 90% of groundwater supplies in the USA have silicon concentrations in the range 7 to 43 mg SiO₂ l⁻¹, whereas sulphate shows a variability eight times greater.

Many major constituents in river water show an inverse relationship between concentration and discharge². This is normally thought to be caused by dilution of baseflow by storm runoff. In the case of silicon the concentration in solution is often insensitive to change in discharge compared with other major components. For example, of seven US rivers cited by Davis^{5,6}, six show no discernible change in dissolved silicon with discharge, and only one (Anchor River, Alaska) exhibits a dilution with increased river flow. A similar lack of correlation was found in studies of five small catchments in the Swiss Pre-alps⁷. Gibbs⁸ showed that in the Amazon silicon levels of about 9 to 12 mg SiO₂ l⁻¹ were

maintained in spite of considerable seasonal change in discharge. For a small catchment in Maryland, dissolved silicon decreased by 10% (9.4 to 8.4 mg SiO₂ l⁻¹), through a storm event⁹, although the stream discharge increased by 284%. Detailed sampling in the Hubbard Brook experimental catchments¹⁰ showed that, although levels of dissolved silicon were inversely related to discharge, the slope of the regression line was considerably less than that expected from dilution of baseflow by rainwater containing a negligible amount of silicon. A study of rivers in Norfolk, England¹¹, shows that, except in periods of high plant productivity, dissolved silicon varied within very narrow limits (10 to 12 mg SiO₂ l⁻¹) although the discharge showed much larger variations.

These results indicate that relative to other major dissolved constituents, river water silicon tends to be remarkably uniform on a worldwide basis and shows little response to change in discharge. They suggest that some controlling mechanism exists for silicon in natural waters which does not apply to other major components. Diatoms and other plankton can vastly deplete dissolved silicon, for example to less than 1 mg SiO₂ l⁻¹ during the spring bloom in some Norfolk rivers¹¹. Silicon is also stored in some catchments by terrestrial macrophytes. But neither of these biological mechanisms seems adequate to maintain near constant concentration through the rapidly changing discharge of a storm hydrograph or to explain the small global inter-river variability.

An abiological buffering mechanism seems more likely. Dissolution of silicate minerals, although the ultimate source of most silicon in solution, is unlikely to be sufficiently rapid in terms of individual hydrographic events. Davis⁶ suggests that desorption of silicic acid from soil particles is more likely. In laboratory experiments¹² hydrated oxides of aluminium, iron, manganese and magnesium can adsorb silicon from dilute solutions (3 mg SiO₂ l⁻¹). The cycle of silicon in some lakes seems to be intimately linked to the formation and dissolution of iron and manganese oxide solid phases, in response to changes in redox potential^{13,14}. Reactions of this type may explain the large scale removal of dissolved silicon, above that accounted for by biological processes, found in Lake Biwa, Japan¹⁵.

The behaviour of dissolved phosphorus seems to be similar in many ways to that of silicon. Deoxygenation of lake sediments results in the release of both elements¹³, and in the Amazon basin rapid equilibrium is established between sediments and dissolved phosphorus in the river water¹⁶.

For soils, there is laboratory evidence that levels of dissolved silicon are controlled by sorption reactions involving oxides of iron and aluminium^{17,18}. These studies, which have used both natural soils and pure solid phases, show that both adsorption and desorption are possible. Beckwith and Reeve¹⁸ suggest that for soil waters much rapidly soluble silicon is derived from sorption sites in the soil and not from solution of specific silicon compounds.

Although dissolution of solid phases and biological activity are important, they cannot fully explain the low temporal and spatial variability found for dissolved silicon levels in fresh waters. It seems that sorption reactions involving dissolved silicon and solid phases are a more likely buffer mechanism controlling the concentration of dissolved silicon in rivers, lakes and soils.

A. M. C. EDWARDS
P. S. LISS

School of Environmental Sciences,
University of East Anglia,
Norwich NOR 88C

Received January 11, 1973.

¹ Alexander, G. B., *J. Amer. Chem. Soc.*, 75, 5655 (1953).

² Hem, J. D., *US Geol. Surv. Water-Supply Paper*, No. 1473 (1970).

³ Burton, J. D., Leatherland, T. M., and Liss, P. S., *Limnol. Oceanogr.*, 15, 473 (1970).

⁴ Livingstone, D. A., *US Geol. Surv. Prof. Pap.*, No. 440-G (1963).

- ⁵ Davis, S. N., *Amer. J. Sci.*, **262**, 870 (1964).
- ⁶ Davis, S. N., *Trans. Amer. Geophys. Union*, **50**, 141 (1969).
- ⁷ Keller, H. M., *J. Hydrol. (NZ)*, **9**, 133 (1970).
- ⁸ Gibbs, R. J., *Geochim. Cosmochim. Acta*, **36**, 1061 (1972).
- ⁹ Cleaves, E. T., Godfrey, A. E., and Bricker, O. P., *Geol. Soc. Amer. Bull.*, **81**, 3015 (1970).
- ¹⁰ Johnson, N. M., Likens, G. E., Bormann, F. H., Fisher, D. W., and Pierce, R. S., *Water Resource Res.*, **5**, 1353 (1969).
- ¹¹ Edwards, A. M. C., *J. Hydrol.*, **18**, 219 (1973).
- ¹² Harder, H., *Geochim. Cosmochim. Acta*, **29**, 429 (1965).
- ¹³ Mortimer, C. H., *Limnol. Oceanogr.*, **16**, 387 (1971).
- ¹⁴ Kato, K., *Geochem. J.*, **3**, 87 (1969).
- ¹⁵ Kobayashi, J., in *Chemical Environment in the Aquatic Habitat* (edit. by Golterman, H. L., and Clymo, R. S.), 41 (North-Holland, Amsterdam, 1967).
- ¹⁶ Gessner, F., *Int. Revue Ges. Hydrobiol.*, **45**, 339 (1960).
- ¹⁷ Jones, L. H. P., and Handreck, K. A., *Nature*, **198**, 852 (1963).
- ¹⁸ Beckwith, R. S., and Reeve, R., *Austral. J. Soil Res.*, **2**, 33 (1964).

Grass Roots at the Base of the Neogene

THE history of life on Earth seems to have been punctuated by fairly swift changes in ecosystems, brought about initially by tectonic or cataclysmic events. The flora, being at the base of the food chain, would have a secondary effect on faunal evolution when undergoing even slight modifications. Such an instance may be found at the end of the Mesozoic, when the oncoming angiosperms must have greatly modified faunal ecosystems on the land. But it would be incorrect to assume that these changes had no relevance to seafloor communities, especially with regard to the appearance of grasses.

The Graminae, although appearing in the Late Cretaceous, did not become abundant until early Neogene¹. This group must have played a fundamental role in the shaping of Cainozoic ecosystems by providing a vast ground level food supply capable of being continuously cropped by herds of mammals, whilst also providing niches for many smaller organisms. It must also have modified the geomorphology by stabilization of the soil surface. The Marram grass *Ammophila* is a present day example, where stabilization of coastal dunes has enabled the establishment of complex ecosystems on formerly barren sands. Similar effects follow the appearance of marine grasses such as *Thalassia* in backreef communities in the West Indies². It follows that remarkable changes must have occurred after the initial colonization of the seafloor by the Graminae. Back-reef and inshore sediments would have been stabilized, accompanied by an accumulation of silt and mud-sized particles, an increase in the organic content of sediments and thus of deposit feeding organisms. Marine grasses would also have provided new sources of food for the macrofauna and microfauna and new means of dispersal for benthonic and phytal organisms, the latter perhaps resulting in a decreasing chance of isolation and speciation.

Unfortunately, the date of the appearance of marine grasses is not traceable by palynology because they produce pollen without exine, and so are not fossilized¹. Their former presence can nevertheless be deduced by sedimentology and palaeontology. Sediments from these meadows are characterized by a bimodal distribution of grain size, having abundant trapped silt together with much coarse biogenic material, especially gastropods, serpulids, ostracods and foraminifera. My analysis of foraminifera living on seagrass from the Caribbean³ has shown that they consist largely of different species to those dwelling on the sediment and are therefore useful as palaeoecological indicators. Examination of the published fossil record suggests that this seagrass association occurred for the first time in the early Miocene Alum Bluff stage of Florida, the microfauna of which has been described by Puri^{4,5}. It was also at this time that the typically phytal foraminifera *Sorites*, *Amphisorus* and *Marginopora* first appeared, becoming widespread throughout the tropics. On the debit side, the Miocene witnessed the extinction of the larger orbitoid and nummulitid foraminifera. This event may

also have been precipitated by the appearance of seagrass, for they were probably adapted to the less stable sediments of Palaeogene reefal areas.

It therefore seems credible that the changes from Palaeogene to Neogene benthonic biofacies can be partly attributed to the effects that encroaching marine grasses must have had on the ecology.

I thank Professor T. Barnard and the Geology Department of University College, London, for help and advice, the Royal Navy for logistic support and the Natural Environment Research Council for financial support.

M. D. BRASIER

Department of Palaeontology,
Institute of Geological Sciences,
Ring Road, Halton,
Leeds LS15 8TQ

Received April 19, 1973.

- ¹ Tschudy, R. H., and Scott, R. A., *Aspects of Palynology* (Wiley, London, 1969).
- ² Scoffin, T., *J. Sediment. Petrol.*, **40**, 249 (1970).
- ³ Brasier, M. D., thesis, Univ. London (1972).
- ⁴ Puri, H. S., *Bull. Florida Geol. Surv.*, **36** (1953).
- ⁵ Puri, H. S., *J. Palaeont. Soc. India*, **1**, 153 (1956).

BIOLOGICAL SCIENCES

Synthesis of a Matrix-supported Enzyme in Non-aqueous Conditions

IN recent years the chemical and physical properties of matrix-supported enzymes have received considerable attention in the chemical literature^{1,2}. The discovery that biologically active compounds can be fixed artificially to insoluble polymeric supports has generated an interest in these materials which is manifest in their wide applicability to the study of complex biological systems. This is so because, in general, the immobilization of active proteins has been shown to produce several significant chemical and physical changes in the protein relative to its native-state counterpart. For example, certain enzymes, which in an isolated extracellular environment exhibit relatively low thermal stability, can be materially stabilized by attachment to an inert polymeric support. Furthermore, chemical properties, which are manifest in the mechanism of action, can be altered significantly by the technique, for example reaction kinetics and substrate specificity.

Applied research ranges from the study of matrixized enzymes as *in vitro* models for complex *in vivo* systems to, for example, their more practical use in continuous assay systems for glucose, urea and anticholinesterase compounds³. The uses of matrix-supported enzymes in the continuous resolution of racemic mixtures and continuous isomerizations and degradations of saccharides have become important industrial processes⁴.

In general, four basic procedures are used for preparing immobilized enzymes: (1) adsorption on synthetic resins; (2) entrapment in gel lattices; (3) covalent binding through nucleophilic groups of the protein with electrophilic functional groups on inert polymers, and (4) covalent cross-linking with certain bifunctional reagents. Moreover, most recent research dealing with the synthesis of insolubilized enzymes has emphasized procedures which effect protein attachment to the support through covalent bonds. Typically, such synthetic routes as azide displacement, diazo coupling, and binding through cyclic imidocarbonates are used^{2,5,6}. Invariably though, the choice of activation procedure for a given polymeric support has been made so as to be consistent with covalent bond formation in aqueous

media only. Obviously, this condition places a severe restriction on the design of chemical reactions by which the protein is covalently bound to the support. As a result, the many chemical techniques available to the synthetic chemist have not been utilized in the preparation of matrix-supported enzymes. One purpose of the study reported here was to determine if this "aqueous restraint" was justified or only assumed as part of an historical artefact, since biologically active materials are typically studied in aqueous (buffered) media, ostensibly to mimic their natural environment*.

Our approach to the first non-aqueous synthesis of a matrix-supported enzyme is outlined in Fig. 1. Because enzymes (for example, lysozyme) typically have a low solubility in organic solvents it was convenient to perform the coupling reaction using a support which could first be dissolved in an appropriate organic solvent, then activated to yield a soluble matrix containing the reactive species, thus binding the insoluble proteins in a two phase reaction system. For this reason, the linear 4-vinylbenzoic acid-styrene copolymer¹⁰ (I) was chosen as the support. (All attempts to prepare a homopolymer of 4-vinylbenzoic acid¹¹ yielded only very low molecular weight polymers, which did not possess solubility characteristics consistent with the method outlined in Fig. 1.) The copolymer prepared in a 1:3 molar ratio from the monomers displayed solubility characteristics consistent with final isolation of the polymer-enzyme complex by a non-solvent precipitation process. The scheme outlined in Fig. 1 allowed complete removal of all unreacted enzyme before product precipitation and purification, thus avoiding enzyme adsorption to the precipitated polymer-enzyme complex. The model enzyme—lysozyme—was chosen primarily because of its ready availability in highly purified form. *N,N'*-carbonyldiimidazole was chosen as the coupling reagent for two reasons. First, it has been shown to be very effective in peptide synthesis^{12,13}, reacting rapidly and essentially quantitatively with carboxylic acids at room temperature to yield intermediate amides (Fig. 1). Coupling with amino (or alcoholic groups)^{12,13} can then proceed by displacement of imidazole. Second, the by-products of the coupling procedure are innocuous.

The experimental procedure was as follows: a copolymer of styrene (4.22 g; 0.0405 mol) and 4-vinylbenzoic acid¹⁴ (2.00 g; 0.0135 mol) was prepared by treating a mixture of the monomers with 50 mg of benzoyl peroxide at 80° C. After 24 h a clear, colourless, hard polymer formed which was removed from the polymerization vessel, ground to a fine powder, and used without further purification. For the enzyme binding reaction, 200 mg of copolymer was dissolved in 5 ml of freshly distilled *N,N*-dimethylformamide. *N,N'*-carbonyldiimidazole^{12,13} (70 mg; equivalent to the amount of *p*-carboxystyrene contained in the copolymer assuming a statistical distribution of the monomer in the copolymer) was added and the solution was stirred for 30 min at room temperature. Crystalline egg-white lysozyme (20 mg) was added in one portion and the mixture was stirred for 24 h at room temperature. At the end of the stirring period, unreacted lysozyme was still visible in the mixture. The latter was removed by centrifugation and the organic supernatant was separated. At this point in the reaction sequence, it was frequently noted that slight cooling of the dimethylformamide reaction mixture (15° C) resulted in precipitation

of the polymer-enzyme complex. This occurred in cases where the concentration of the product was more than about 40 mg ml⁻¹ in dimethylformamide solution. Similar reactions carried out on polymer solutions containing enzyme, however, without added *N,N'*-carbonyldiimidazole, failed to form precipitates even at 5° C. Polymer solutions at 40 mg ml⁻¹ concentration treated with *N,N'*-carbonyldiimidazole, but not enzyme, likewise gave no precipitation when cooled. These observations are consistent with the expected large increase in molecular weight as the coupling reaction proceeds. The copolymer-enzyme complexes were finally isolated by dilution of the dimethylformamide solution into water or buffer (pH 6.2 phosphate, 0.2 M). White granular precipitates were thus obtained, subsequently removed by filtration, and washed thoroughly with buffer to remove the last traces of organic solvent.

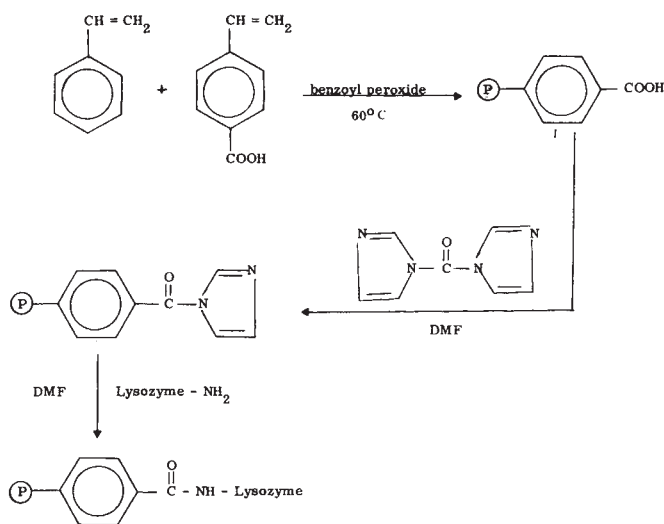


Fig. 1 Non-aqueous synthesis of matrix-supported lysozyme. DMF, Dimethylformamide.

The amount of protein bound to the polymer was estimated by the method of Bramhall *et al.*¹⁵. Typically, 5–7 mg of lysozyme could be bound to 200 mg of copolymer. The activity of the bound protein toward *Micrococcus lysodeikticus* was determined by the method of Shugar¹⁶ and is expressed in spectrophotometric units (SU) as follows:

$$SU = \frac{\Delta A/\text{min} \times 1,000}{\text{mg protein}}$$

Native lysozyme exhibited an activity of 10,500 SU, whereas the matrix-supported protein gave an activity of 4,330 SU. Thus, on a weight basis, the matrix-supported material retains about 40% of native activity.

The fact that the enzyme "survived" the application of a rigorous chemical synthesis in anhydrous conditions is significant for numerous reasons. In the first instance, the manipulation of enzymes in mixtures of water and organic solvents is known to result in an "unfolding" of the enzyme in a way which exposes the hydrophobic portion of the molecule¹⁷. The latter effect may be minimal in the present case since the protein was not in solution, but by judicious choice of organic solvent parameters, the degree of unfolding might well be controlled, thus allowing subsequent binding while the protein assumes various predetermined and variable conformations. In those conditions it should be possible to design appropriate chemical syntheses to allow a highly selective chemical binding process to occur, in fact, one involving hydrophobic groups preferentially in the presence of hydrophilic groups. We believe that

* The literature contains a few cases where proteins have been subjected to chemical treatment in anhydrous conditions. For example, several biologically active proteins have been shown to be both soluble in and recoverable from anhydrous hydrogen fluoride⁷ or anhydrous trifluoroacetic acid⁸ without loss of enzymatic activity. Also, Siegel and Roberts⁹ have briefly reported the activities of the redox enzymes peroxidase and catalase in a number of anhydrous organic solvents. The feasibility of carrying out chemical reactions for the immobilization of proteins in strictly anhydrous media has, however, not been examined to our knowledge.

these properties can be exploited to both theoretical and practical advantage.

This research was supported by the National Science foundation. We thank Mr Charles W. Barker for technical assistance.

GREG J. BARTLING
HARRY D. BROWN
SWARAJ K. CHATTOPADHYAY

Cancer Research Center,
Columbia,
Missouri 65201

Received December 18, 1972.

- ¹ Brown, H. D., and Hasselberger, F. X., in *Chemistry of the Cell Interface*, Part B (edit. by Brown, H. D.), 7, 185 (Academic Press, New York, 1971).
- ² Stark, G. R. (edit.), *Biochemical Aspects of Reactions on Solid Supports* (Academic Press, New York, 1971).
- ³ Dixon, M., and Webb, E. C., *Enzymes* (second ed.) (Academic Press, New York, 1964).
- ⁴ Melrose, J. H., *Rev. Pure and Appl. Chem.*, **21**, 83 (1971).
- ⁵ Bartling, G. J., Brown, H. D., Forrester, L. J., Koes, M. T., Mather, A. N., and Stasiw, R. O., *Biotech. Bioeng.* (in the press).
- ⁶ Axen, R., Porath, J., and Ernback, S., *Nature*, **214**, 1302 (1967).
- ⁷ Katz, J. J., *Nature*, **173**, 265 (1954).
- ⁸ Katz, J. J., *Nature*, **174**, 509 (1954).

- ⁹ Siegel, S. M., and Roberts, K., *Space Life Sci.*, **1**, 131 (1968).
- ¹⁰ Letsinger, R. L., Kornet, M. J., Mahadevan, V., and Jerina, D. M., *J. Amer. Chem. Soc.*, **86**, 5163 (1964).
- ¹¹ Marvel, C. S., and Overberger, C. G., *J. Amer. Chem. Soc.*, **67**, 2250 (1945).
- ¹² Paul, R., and Anderson, G. W., *J. Amer. Chem. Soc.*, **82**, 4596 (1960).
- ¹³ Paul, R., and Anderson, G. W., *J. Org. Chem.*, **27**, 2094 (1962).
- ¹⁴ Leebrick, J. R., and Ramsden, H. E., *J. Org. Chem.*, **23**, 935 (1958).
- ¹⁵ Bramhall, S., Noack, N., Wu, M., and Loewenberg, J. R., *Anal. Biochem.*, **31**, 146 (1969).
- ¹⁶ Shugar, D., *Biochim. Biophys. Acta*, **8**, 302 (1952).
- ¹⁷ Ghosh, S., Breese, K., and Bull, H. B., *J. Colloid. Sci.*, **19**, 457 (1964).

Rate Constants of Electron Transfer Processes in Solution: Dependence on the Redox Potential of the Acceptor

ONE-ELECTRON oxidation-reduction reactions have been established in several enzymatic reactions, and electron transport has been found to be necessary in phosphorylation and mito-

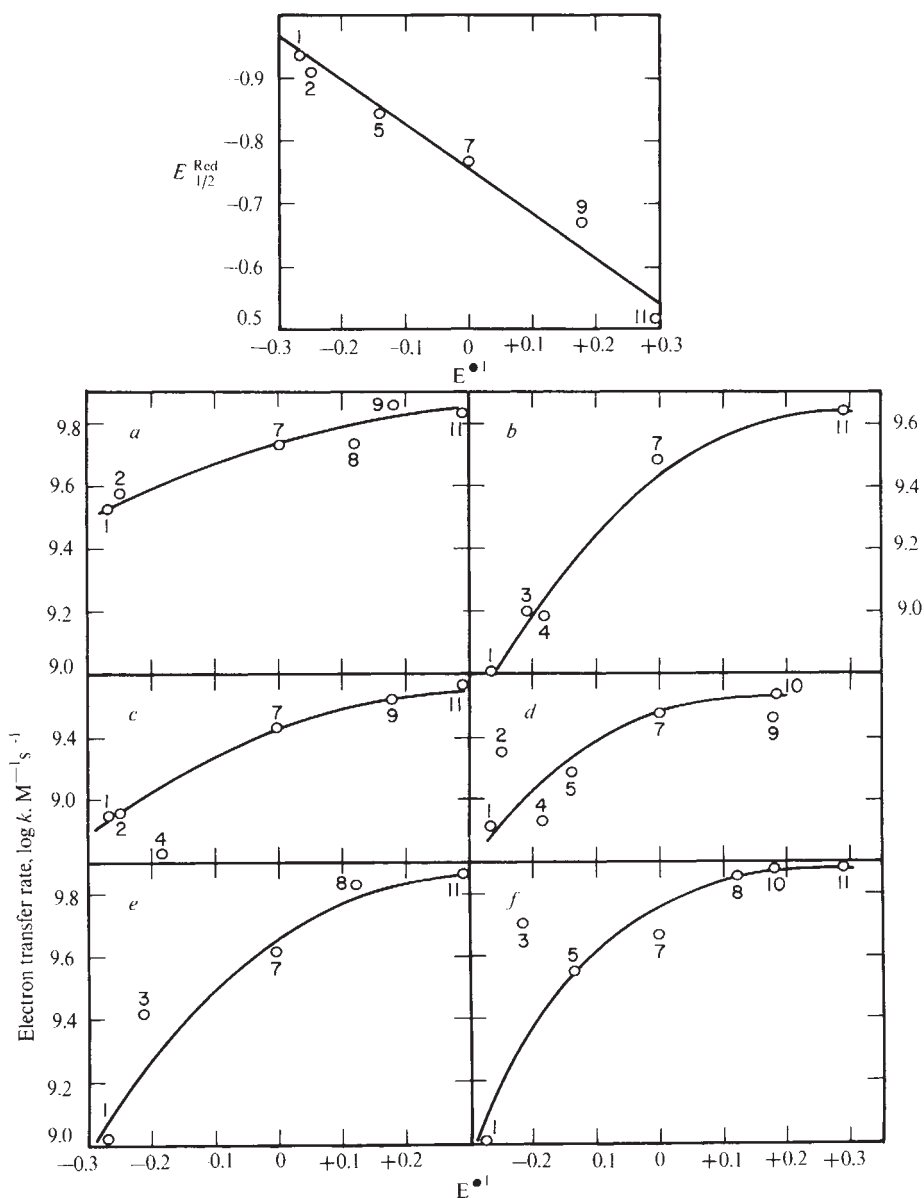


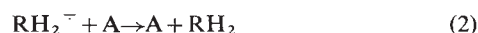
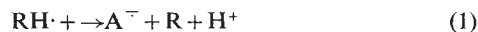
Fig. 1 Dependence of $\log k$, the electron transfer rate constants of various free radicals (produced by reaction of substrate with e_{aq}^-) upon the $E^{\bullet 1}$ values of the acceptors. The numbers refer to the acceptor used. Included is the correlation between $E^{\bullet 1}$ and the half-wave reduction potential of some of the acceptors (taken from ref. 17). a, Nicotinamide, pH 7.0; b, NAD^+ , pH 6.0; c, N-ethyl maleimide, pH 6.0; d, acetate, pH 9.2; e, Co^{2+} , pH 7.0; f, Cd^{2+} , pH 7.0.

chondrial systems¹⁻⁴. The role of semiquinone radicals as active agents in biochemical electron transfer reactions has been indicated^{1,2}, and correlations between the redox potential of the quinones and their effectiveness in catalysing a particular type of phosphorylation reaction have been suggested^{1,2,5}. This principle has never been demonstrated quantitatively by chemical kinetics.

The redox reactions of a number of organic free radicals against menaquinone as acceptor have been studied⁶⁻¹⁰. The rate constants k for electron transfer from some of these radicals to different acceptors as a function of the redox potential ($E^{\bullet 1}$) of the acceptors is the subject of the study described here. The fast-reaction technique of pulse radiolysis was used^{10,11} for generating the free radicals in water (a) by reaction of OH radicals with some substrates (in the presence

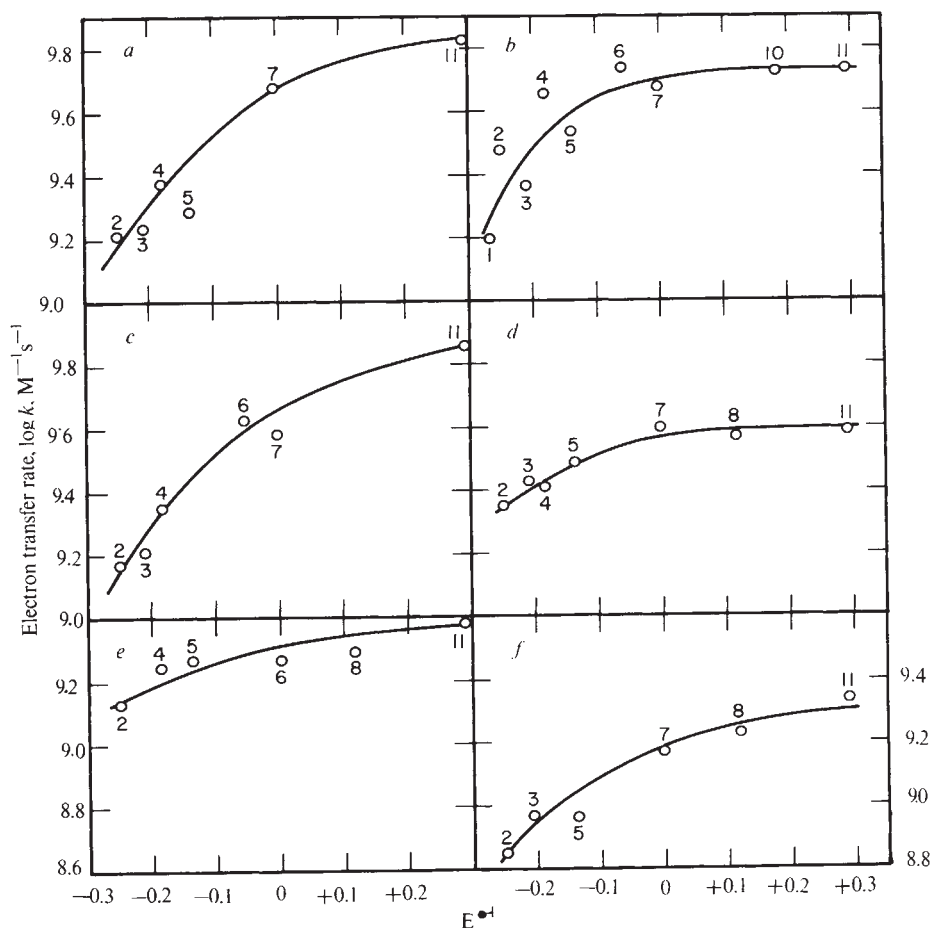
ferricyanide (⑥, -0.055), menaquinone (⑦, 0.002), 1,4-naphthaquinone-2-sulphonate (⑧, 0.118), 2,5-dimethyl-*p*-benzoquinone (⑨, 0.176), MnO_4^- (⑩, 0.180), and *p*-benzoquinone (⑪, 0.293). The $E^{\bullet 1}$ values ($\text{pH}=7.0$, 25°C) given in parenthesis were from ref. 14.

The electron transfer reactions taking place can be represented as:



The $\text{p}K_a$ of the semiquinones^{10,15,16} used lie in the range 4–5.0: $\text{AH} \rightleftharpoons \text{A}^\bullet + \text{H}^+$. The results are presented in Table 1 and Figs 1 and 2. In addition, the k values of CH_2OH ,

Fig. 2 Dependence of $\log k$, the electron transfer rate constants of various free radicals (produced by reaction of substrate with OH radicals) on the $E^{\bullet 1}$ values of the acceptors. a, Formate, pH 7.0; b, isopropyl alcohol, pH 7.0; c, cytosine, pH 7.0; d, glycine, pH 8.0; e, triglycine, pH 10; f, glycolic acid, pH 7.0.



of N_2O to convert e_{aq}^- into OH radicals), (b) by reaction of e_{aq}^- with other substrates (in argon and $\sim 1.0\text{ M}$ *t*-butyl alcohol to scavenge⁹ the OH radicals).

The acceptor concentration was $\sim 5 \times 10^{-5}\text{ M}$ for quinones and $\sim 1 \times 10^{-4}\text{ M}$ for other acceptors. The [radical] was kept at $\geq 5\%$ [acceptors]. The k values were determined at $\sim 22^\circ\text{C}$ by following (a) the formation kinetics of the semiquinone radical anions of the quinones (at 400 nm) and of riboflavin (at 560 nm¹³); (b) the disappearance kinetics of MnO_4^- at 545 nm and of ferricyanide at 420 nm. From the pseudo-first order rates, the second order rates were determined. The following acceptors were studied: 9,10-anthraquinone (①, -0.266), 9,10-anthraquinone-2-sulphonate (②, -0.250), riboflavin (③, -0.208), 9,10-anthraquinone-2,6-disulphonate (④, -0.184), 2-hydroxy-1,4-naphthaquinone (⑤, -0.139),

$(\text{CH}_3)_2\dot{\text{C}}\text{OH}$, $\text{NH}_2\dot{\text{C}}\text{HCOO}^-$ and Cd^+ to NAD^+ ($E^{\bullet 1} = -0.320$) as an acceptor were found to be 1.0×10^9 , 1.0×10^9 , 1.5×10^9 and $2.9 \times 10^9\text{ M}^{-1}\text{ s}^{-1}$, respectively.

From the experimental results (rate values $\pm 10\%$): (a) a quantitative correlation appears between the k values from many free radicals and the $E^{\bullet 1}$ of various electron acceptors (quinones, riboflavin, NAD^+ , ferricyanide and MnO_4^-); (b) the rate is expected to increase with increase in $\Delta E^{\bullet 1}$ between the donor radical and the acceptor (as indicated by the different slopes for the various radical donors); (c) the half-wave reduction potential $E_{1/2}^{\text{red}}$ of the radical is probably a better parameter to use but only limited data are available¹⁷; there is a direct correlation, however, between $E^{\bullet 1}$ and $E_{1/2}^{\text{red}}$ (see Fig. 1), as well as between the electron affinity and $E_{1/2}^{\text{red}}$ (ref. 18); (d) as there is a good linear relationship between the

Table 1 Rate Constants of One-Electron Oxidation-reduction Reactions of Free Radicals with Various Acceptors in Aqueous Solution

Substrate	Donor radical	Rate of electron transfer, $k \times 10^{-9} \text{ M}^{-1} \text{ s}^{-1} *$										
		①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩	⑪
		-0.266	-0.250	-0.208	-0.184	-0.139	-0.055	+0.002	+0.118	+0.176	+0.180	+0.293
Formate	$\cdot\text{CO}_2^\ddagger$	—	1.65	1.71	2.40	1.95	†	4.80	†	—	†	6.60
Isopropyl alcohol	$(\text{CH}_3)_2\dot{\text{C}}\text{OH}^\ddagger$	1.62	3.04	2.30	4.60	3.40	5.55	4.76	†	—	4.24	5.40
Cytosine	$\cdot\text{C-OH}^\ddagger$	—	1.48	1.61	2.26	†	4.29	3.80	†	—	†	7.20
Triglycine	$\text{H}_2^+-\text{Gly-Gly-NH}\dot{\text{C}}\text{HOO}^\ddagger$	—	1.36	—	1.79	1.88	—	1.88	1.97	—	—	2.46
Glycine	$\text{NH}_2\dot{\text{C}}\text{HCOO}^\ddagger$	—	2.25	2.72	2.56	3.12	†	4.00	3.30	—	†	3.90
Glycollic acid	$\text{OH}\dot{\text{C}}\text{HCOO}^\ddagger$	—	0.71	0.93	—	0.91	—	1.47	1.69	—	†	2.20
Nicotinamide	$\cdot\text{NH}^\S$	3.50	3.80	—	—	†	—	5.40	5.45	7.25	—	7.00
NAD ⁺	$\cdot\text{NAD}^\S$	0.41	—	1.00	0.96	—	—	3.10	—	†	—	4.40
N-ethyl maleimide	$\cdot\text{NEM}^\S$	0.83	0.82	—	0.46	—	—	3.00	—	4.40	—	5.50
Acetoacetate	$\text{CH}_3\dot{\text{C}}(\text{O}^-)\text{CH}_2\text{COO}^\S$	0.67	2.10	†	0.72	1.53	0.73	3.70	†	3.34	4.80	†
Cobalt sulphate	$\text{Co}^+^\S$	1.05	†	2.55	†	†	†	4.10	6.83	†	†	7.35
Cadmium sulphate	$\text{Cd}^+^\S$	1.03	†	5.10	†	3.57	†	4.68	7.35	†	7.80	7.70

* See text for acceptor corresponding to each number. $E^{\circ 1}(\text{pH } 7.0)$ used as the redox potential.

† Slow thermal reaction occurring in this system.

‡ Donor radical produced by reaction of substrate with OH radicals.

§ Donor radical produced by reaction of substrate with e_{aq}^- .

redox potential and the energy of the highest occupied orbital¹⁹ (calculated by LCAO-MO method) one electron should be removed (or added) from that orbital in the rate determining step.

In conclusion, these results show that, to a first approximation, the rate constants for electron transfer processes from free radicals to various acceptors are proportional to the redox potential of the acceptor. As redox reactions are used extensively to introduce or withdraw electrons in biochemical reactions, such correlations can be used to predict the course of various competitive reactions, as well as to derive an approximate $E^{\circ 1}$ value for a compound.

P. S. RAO
E. HAYON

Pioneering Research Laboratory,
US Army Natick Laboratories,
Natick, Massachusetts 01760

Received January 8, 1973.

¹ *Electron and Coupled Energy Transfer in Biological Systems* (edit. by King, T. E., and Klingenberg, M.), 1, parts A and B (Dekker, New York, 1971 and 1972).

² *Biochemistry of Quinones* (edit. by Morton, R. A.) (Academic Press, New York, 1965).

³ Taube, H., *Electron Transfer Reactions of Complex Ions in Solution* (Academic Press, New York, 1970).

⁴ Reynolds, W., and Lumry, R., *Mechanisms of Electron Transfer Reactions* (Ronald Press, New York, 1966).

⁵ Trebst, A., and Eck, H., *Z. Naturforsch.*, **16b**, 44 (1961).

⁶ Simic, M., and Hayon, E., *Int. J. Radiat. Biol.*, **20**, 589 (1971).

⁷ Hayon, E., and Simic, M., *Radiat. Res.*, **50**, 464 (1972).

⁸ Simic, M., and Hayon, E., *Int. J. Radiat. Biol.*, **22**, 507 (1972).

⁹ Hayon, E., and Simic, M., *J. Amer. Chem. Soc.* (in the press).

¹⁰ Rao, P. S., and Hayon, E., *Biochim. Biophys. Acta* (in the press).

¹¹ Simic, M., Neta, P., and Hayon, E., *J. Phys. Chem.*, **73**, 3794 (1969).

¹² Keene, J. P., Black, E. D., and Hayon, E., *Rev. Sci. Instrum.*, **40**, 1199 (1969).

¹³ Land, E. J., and Swallow, A. J., *Biochemistry*, **8**, 2117 (1969).

¹⁴ *Handbook of Biochemistry*, J. 33 (Chemical Rubber Co., 1970).

¹⁵ Hayon, E., Ibata, T., Lichtin, N. N., and Simic, M., *J. Phys. Chem.*, **76**, 2072 (1972).

¹⁶ Willson, R. L., *Chem. Comm.*, 1249 (1971).

¹⁷ Peover, M. E., *J. Imp. Coll. Chem. Soc.*, 4540 (1962).

¹⁸ Briegleb, G., *Angew. Chem.*, **3**, 617 (1964).

¹⁹ Fueno, T., Lee, T., and Eyring, H., *J. Phys. Chem.*, **63**, 1940 (1959).

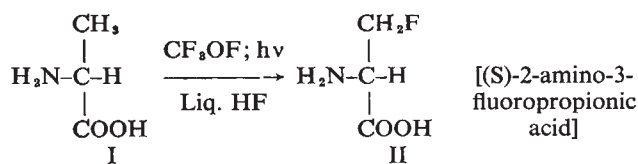
New Antibacterial Agent via Photofluorination of a Bacterial Cell Wall Constituent

We wish to report that a highly active antibacterial agent with novel properties, which is active against gram-negative as well as gram-positive bacteria, is generated by C-fluorination of a key component of the bacterial cell wall.

We proposed C-fluorination of bio-active molecules via photofluorination by fluoroxytrifluoromethane¹ for creation of agonists and antagonists of biologically important compounds². This direct method of $-\text{CH}- \rightarrow -\text{CF}-$ transformation appears successful in creating highly effective specific inhibitors of enzymes³ via C-fluorination of properly selected bio-active molecules¹.

D-Alanine is a ubiquitous component of the bacterial cell wall⁴; it is endogenously synthesized by the bacteria, but, unlike L-alanine, it plays no essential role in human metabolism⁵. Further, the mechanism of antibacterial action of the penicillins, cephalosporins, and cycloserine has been shown to involve inhibition of various enzyme systems related to biosynthesis or utilization of the D-alanine component of the peptidoglycan of the bacterial cell wall⁶⁻⁸. Thus, it was thought that C-fluorination of D-alanine might generate a specific antimetabolite with antibacterial activity.

Photofluorination¹ of D-alanine with CF_3OF in liquid HF solvent produced 3-fluoro-D-alanine (II)—in better than 50% yield. Isolation was by ion-exchange chromatography on 'Dowex-X8' resin column, eluting with 1 M HCl. The compound was crystallized from water and had an m.p. of 168° C (dec.).



The assigned structure is in agreement with: (a) PMR spectrum ($\text{D}_2\text{O}/\text{DCl}$): two triplets (CH_3) centred at 5.3 p.p.m. ($J_{\text{H-F}}$: 60 Hz); two multiplets (CH) centred at 4.65 p.p.m. (5.02 and 4.28 p.p.m.). (b) F^{19} NMR spectrum ($\text{D}_2\text{O}/\text{DCl}$): two

triplets centred at +231.2 p.p.m. and +231.8 p.p.m. (External CFCl₃: O). (c) Optical Rotatory Dispersion (in 1 M HCl): [α]₂₄₄: -1,410° (— trough); [α]₂₁₀: O (cross-over). (For D-alanine: [α]₂₂₄: -1,830/— trough) [α]₂₅: -10° (c, 3 in 1 M HCl). (d) In the Spinco-Beckman Automatic Amino Acid analyser II gave a single, symmetrical peak. (e) Calculated for C₃H₆NO₂F: C, 33.65; H, 5.65; N, 13.08; F, 17.74%. Found: C, 33.63; H, 5.87; N, 13.03; F, 17.32%.

When present at 100 μ g ml.⁻¹ in bacteriological culture media commonly used for antibiotic susceptibility testing, II failed to inhibit growth of a majority of the strains of bacterial pathogens against which it proved highly effective *in vivo*. For example, among the strains listed in Table 2, only *Streptococcus pyogenes* and *Diplococcus pneumoniae* were inhibited, and in subsequent titrations performed in brain heart infusion were found to have minimum inhibitory concentrations of 6 and 13 μ g ml.⁻¹, respectively. Because antimetabolite action is often reversed in complex media, titrations were repeated in a minimal medium⁹ that supported growth of *Escherichia coli* 2017. Inhibition of bacterial growth at low concentrations of II and the predicted reversal of this effect by D-alanine is demonstrated in Table 1. Comparable observations of marked activity in minimal media over complex broths¹⁰ and of antagonism by low proportions of D-alanine¹¹ are reported for D-cycloserine, a well-established inhibitor of the biosynthesis and subsequent conversion of D-alanine into bacterial murein¹².

Table 1 Inhibition by II of Bacterial Growth

Fixed component	Varied component	Concentration, μ g ml. ⁻¹							
—	II	100	50	25	13	6	3	1.5	0
II	—	—	—	—	—	—	+	+	+
50 μ g ml. ⁻¹	D-alanine	+	+	+	+	—	—	—	—
II	—	—	—	—	—	—	+	+	+
25 μ g ml. ⁻¹	D-alanine	+	+	+	+	+	—	—	—

An inoculum of 10⁸ cells ml.⁻¹ of *E. coli* 2017 was added to Davis minimal medium⁹ containing serial dilutions of II alone or a serial dilution of D-alanine in the presence of fixed levels of the anti-bacteria. Following 16 h incubation at 37° C, tubes showing turbidity (>10⁷ cells ml.⁻¹) were scored "+".

Table 2 *In Vivo* Efficacy of II in Mouse

Strain*	Route†	ED ₅₀ mg kg ⁻¹ †
<i>E. coli</i> 2017	SC	18
	PO	19
<i>Klebsiella pneumoniae</i> "B"	PO	5.5
<i>Salmonella schottmuelleri</i> 3010	PO	17
<i>Streptococcus pyogenes</i> 3009	SC × 2	1.1
<i>Diplococcus pneumoniae</i> I-37	SC × 2	5
<i>Staphylococcus aureus</i> 2949	SC	27

* Intraperitoneal challenge averaging 10 LD₅₀.

† Basis for calculating this median protective dose and further details are found in ref. 13.

‡ Therapeutic administered immediately following infection, either subcutaneously (SC) or perorally (PO) in a single dose except where marked (treatment was repeated 6 h following challenge).

According to Table 2, the absolute potency of II is within the range of titrations from these laboratories with chloramphenicol and tetracycline administered by comparable routes in the indicated infections¹³. The new antibacterial agent conferred protection over a wide spectrum of gram-positive and gram-negative pathogens. II has the desirable property of undiminished efficacy by the oral route and a low order of acute toxicity. For example, mice survived a single, oral dose of 2 g kg⁻¹. A unique finding was that *in vivo* protection declined in some infections from 100% (achieved in all

cases at 100 mg kg⁻¹ doses) to as low as 20% by 250 mg kg⁻¹. It is to be noted that 3-fluoro-L-alanine—obtained by photo-fluorination of L-alanine—was at least 10 times more toxic than the D-isomer, being lethal to mice at 250 mg kg⁻¹.

The observation that at fixed high concentrations II is inactive *in vitro* against many bacterial pathogens would scarcely have predicted the high order of *in vivo* efficacy against these same bacterial pathogens. The unique properties of II that account for both this *in vitro* anomaly and the related phenomenon of declining protection at high, but non-toxic, dose ranges observed in therapeutic trials are being actively investigated in these laboratories.

J. KOLLONITSCH

L. BARASH

Merck Sharp & Dohme Research Laboratories,

F. M. KAHAN

H. KROPP

Merck Institute for Therapeutic Research,

Merck & Co., Inc.,

Rahway, New Jersey 07065

Received January 24, 1973.

¹ Kollonitsch, J., Barash, L., and Doldouras, G. A., *J. Amer. Chem. Soc.*, **92**, 7494 (1970).

² Kollonitsch, J., Barash, L., and Doldouras, G. A., *162nd Meeting of the Amer. Chem. Soc.*, Abst. F19 (Washington, 1971).

³ Goldman, P., *Science*, **164**, 1123 (1969).

⁴ Rogers, H. J., and Perkins, H. R., *Cell Walls and Membranes*, **214**, 220, 261 (Spon Ltd, London, 1968).

⁵ Corrigan, J. J., *Science*, **164**, 142 (1969).

⁶ Strominger, J. L., *Biosynthesis of Bacterial Cell Walls*, in *The Bacteria* (edit. by Gunsalus, I. C., and Stanier, R. Y.), **3**, 413 (Academic Press, London, 1962).

⁷ Osborn, M. J., *Ann. Rev. Biochem.*, **38**, 501 (1969).

⁸ Strominger, J. L., *The Harvey Lectures*, Series 64, 179, 190 (Academic Press, New York, 1970).

⁹ Davis, B. D., and Mingioli, E. S., *J. Bact.*, **60**, 17 (1950).

¹⁰ Hoeprich, P. D., *J. Lab. Clin. Med.*, **62**, 657 (1963).

¹¹ Curtis, R., Charamella, L. J., Berg, C. M., and Harris, P. E., *J. Bact.*, **90**, 1238 (1965).

¹² Neuhaus, F. C., in *Antibiotics* (edit. by Gottlieb, D., and Shaw, P. D.), **1**, 40 (Springer, New York, 1967).

¹³ Miller, A. K., Frost, B. M., Valiant, M. E., Kropp, H., and Hendlin, D., in *Antimicrobial Agents and Chemotherapy* (edit. by Hobby, G. L.), **310** (Amer. Soc. Microbiol., Bethesda, Maryland, 1970).

Co-carcinogenic Action of Saccharin in the Chemical Induction of Bladder Cancer

A RAT model for studying the induction of bladder cancer has been developed using intravesicular instillations of N-methyl-N-nitrosourea, MNU¹. A single dose of 2.0 mg MNU into a 150 g Wistar rat acts as an initiator, but does not produce carcinoma unless its action is promoted by further doses. If three further instillations are given, at bi-weekly intervals, all animals develop either transitional or transitional plus squamous cell carcinomas of the bladder epithelium¹.

We used this model to investigate the ability of other compounds to promote to carcinoma the changes initiated by a single, non-carcinogenic dose of MNU. Here we describe the preliminary findings of an experiment, still in progress, to show the effect of dietary saccharin on the response of the bladder to MNU.

Four groups, each of fifty female Wistar rats were fed 41B pelleted diet and water *ad libitum*. The control group, A, did not receive MNU or saccharin. The MNU controls, B, each had a single dose of 2.0 mg MNU instilled through urethral catheter into the bladder at the start of the experiment. The saccharin controls, C, had saccharin added to the drinking water: the animals were weighed and the saccharin content of the water adjusted to maintain an intake of 2.0 g kg⁻¹ body weight d⁻¹ throughout the experiment. The experimental

Table 1

Group	Treatment	Total No. of animals in group	No. killed so far for examination	Time killed	Condition of bladder epithelium
A	None	50	12	Between 9 and 56 weeks	Normal
B	2.0 mg MNU by intravesicular instillation	50	12	Between 3 and 50 weeks	3/12 hyperplastic. All killed 12 weeks after dosing 9/12 normal. Killed between 12 and 50 weeks after dosing
C	2.0 g kg ⁻¹ body weight d ⁻¹ saccharin in drinking water	50	12	Between 9 and 56 weeks	10/12 normal 2/12 mildly hyperplastic. Killed after 36 weeks
D	2.0 mg MNU at week 6 plus 2.0 g kg ⁻¹ body weight d ⁻¹ saccharin in drinking water from week 0	50	12	Between 9 and 56 weeks	1/12 normal 6/12 hyperplastic 5/12 epithelial tumours } see Table 2

group, D, received saccharin by the same method as group C, and each animal had a single dose of MNU 6 weeks after the start of the saccharin regime. The experiment has now been in progress for 56 weeks.

Animals from each group were killed and the histology of the bladders, distended with fixative at necropsy, investigated (Table 1). None of the controls from group A showed any change in the structure of the bladder epithelium. In the first few weeks after receiving a single dose of MNU, the bladder epithelium of animals in group B showed toxic damage followed by hyperplasia. Those killed more than 12 weeks after dosing had a 3 to 4 cell thick epithelium normal in appearance. This transient hyperplastic response of the bladder epithelium to a single dose of MNU has been reported previously¹. Animals from the saccharin control group, C, slowly developed mild hyperplasia and by 56 weeks the epithelium was 4 to 5 cells thick. So far, only twelve animals have been examined from the experimental group D, receiving both saccharin and MNU. In nine of these marked changes of the epithelium were seen, as classified in Table 2. Only three animals showed no greater change than the MNU controls from group B, and two of these, killed at 3 and 10 weeks after MNU respectively, were hyperplastic. The remaining nine all had focal areas of gross hyperplasia in which the epithelium was invaded by blood capillaries (Fig. 1). Of these, five animals also had both papillomatous outgrowths and solid down-growths of transitional cells into the sub-mucosa (Fig. 1), while in three of these animals, the bladder

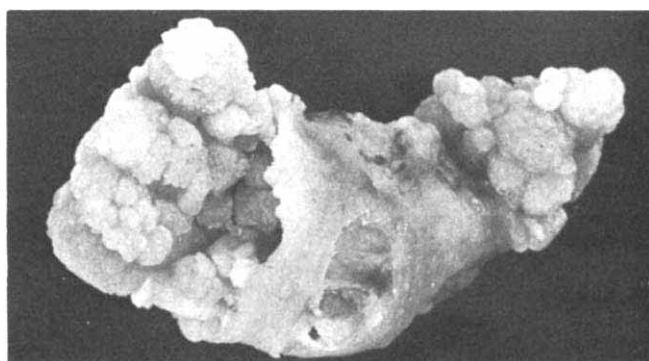


Fig. 2 Gross morphology of bladder from rat which had received 2.0 g kg⁻¹ body weight d⁻¹ saccharin for 56 weeks, plus a single dose of MNU. A mass of epithelial tumours blocks the bladder and extrudes from both cut ends. Part of the external, peritoneal wall of the bladder is seen across the middle of the field. $\times 2.5$.

lumen was partially blocked by gross epithelial tumours (Fig. 2) containing both transitional and squamous cell elements (Fig. 3). Some animals also had calculi in the bladder (Table 1). No calculi were observed in group C female rats on saccharin alone, but about 20% of animals given MNU alone develop calculi without tumour formation.

Table 2 Analysis of Epithelial Changes in 12 out of 50 Animals Killed in Group D

Condition of epithelium	No. of animals	Time killed	No. of animals with calculi in bladder
"Normal" (3 cells thick)	1	20 weeks	0
Mildly hyperplastic (up to 6 cell layers)	2†	3 and 10 weeks	1
Grossly hyperplastic plus invasion of epithelium by blood capillaries	4	12, 13, 16 and 30 weeks	1
Papillary outgrowths and/or polypoid nodular hyperplasia with solid down-growths	2	8 and 24 weeks	2
Invasive carcinoma with both transitional and squamous cell elements	3	21, 50 and 50 weeks	3

* Animals received 2.0 g kg⁻¹ saccharin plus one dose of 2.0 mg MNU after 6 weeks on the saccharin regime.

† These animals showed no greater epithelial hyperplasia than animals killed at the same time from the MNU-only group B, Table 1.

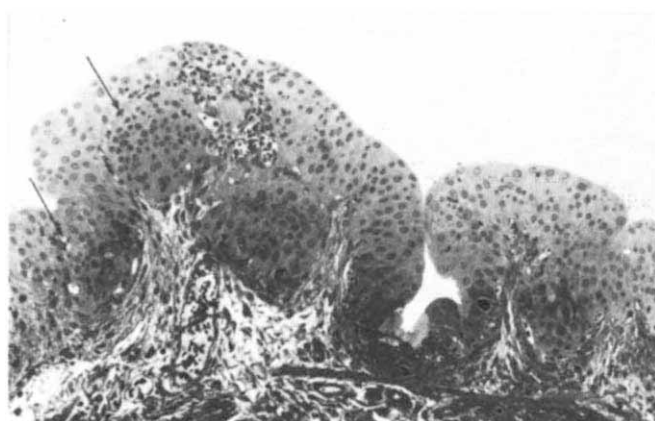


Fig. 1 Bladder epithelium from female rat given 2.0 g kg⁻¹ body weight d⁻¹ saccharin for 24 weeks, plus a single intravesicular dose of 2.0 mg MNU. The epithelium is hyperplastic throughout and has lost its normal cellular differentiation, particularly of the superficial cell layer. Long, branching capillaries extend up into the epithelium (arrows). In this field the epithelium is raised into a small, polypoid, nodular tumour, and there is an adjacent micropapilla. $\times 124$.

It will be approximately a year before all animals are killed and final conclusions can be reached, but the observations obtained so far are of considerable interest. The changes observed in nine out of the twelve animals given saccharin plus MNU indicate that saccharin at this high dose level ($2.0 \text{ g kg}^{-1} \text{ body weight d}^{-1}$) grossly modifies the response of the bladder epithelium to a single dose of MNU.

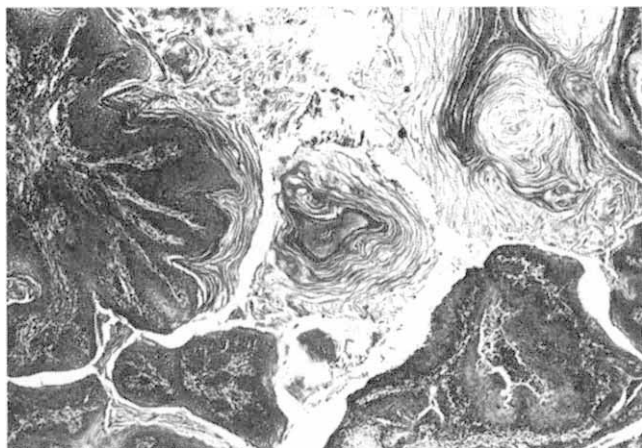


Fig. 3 A section from the bladder shown in Fig. 2. Keratinizing, squamous, epithelial projections partially fill the bladder lumen. Elsewhere, the epithelium is de-differentiated and hyperplastic nodules of transitional cells extend into the submucosa. $\times 37$.

In 1969, cyclamate was withdrawn from the market in the USA on the basis of information supplied to the National Academy of Sciences, National Research Council². Papillary tumours were found in eight out of eighty rats surviving more than seventy-eight weeks fed a 10 to 1 cyclamate to saccharin mixture at a dose rate of $2.5 \text{ g kg}^{-1} \text{ body weight d}^{-1}$. Subsequent work showed that high doses of cyclamate alone produced tumours in a few animals³⁻⁵. Cyclamate and saccharin are usually consumed together by man in a 10:1 ratio mixture. Our experiments show that saccharin is co-carcinogenic in conjunction with a single dose of MNU, and indicate that a promoting action of saccharin with cyclamate and/or other bladder carcinogens is an obvious possibility.

R. M. H. and J. St. J. W. are in receipt of a grant from the Cancer Research Campaign.

R. M. HICKS
J. ST. J. WAKEFIELD
J. CHOWANIEC

School of Pathology,
Middlesex Hospital Medical School,
London W1

Received April 10; revised May 3, 1973.

Lactation, Sebum Excretion and Melanocyte-stimulating Hormone

INCREASED sebum production occurs in patients with breast cancer^{1,2} and this does not seem to be caused by increased androgen production³. The tumour and the sebaceous glands may be responding to the same endocrine drive, and identification of this hormonal drive might be important in the control of breast cancer. To further define the relationship between the mammatrophic and sebotrophic hormones we have studied the effect of suckling on sebum secretion, as suckling is known to stimulate the mammary glands.

We studied forty-six women aged 19–38 yr (mean 27), of whom two had mild acne. Sebum excretion rates (SER) from both sides of the forehead were measured by the method of Strauss and Pochi⁴ as modified by Cunliffe and Shuster⁵ during the last 8 weeks of pregnancy (mean gestation at time of measurement was 38 weeks), and again 8–20 weeks after delivery. Thirteen of the women breast-fed their babies up to post-natal measurement of SER. The other thirty-three either did not breast-feed or did so for a short period only. All the women were delivered of one healthy baby.

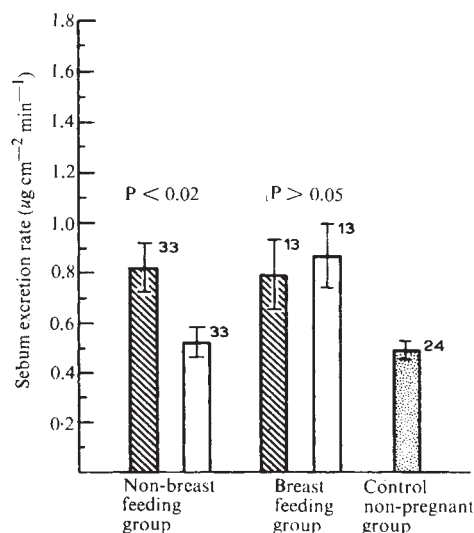


Fig. 1 Sebum excretion rates in non-breast feeding women, breast feeding women and control, non-pregnant women. The number at the top of each column is the number of patients studied in each group. Cross hatched columns, before birth; blank columns, after birth. The mean $\pm$ s.e. is shown at the top of each column.

Sebum was collected in the afternoon to rule out the effect of circadian variation in sebaceous activity⁶. Possible seasonal variations in sebaceous activity were compensated so far as possible by staggering the observations over 2 yr. The patients received no drugs other than laxatives, antacids and haematinics during the pregnancy. Only one woman was taking an oral contraceptive at the time of post-natal measurement of SER and lactation was not suppressed by hormonal or other medication in any of the women.

The SER was also measured in twenty-four non-pregnant control women aged 20–39, none of whom had acne. There was considerable variation in the results from different individuals, and the mean SERs in the various groups are shown in Fig. 1. In women who did not breast-feed, the mean SER during pregnancy ($0.82 \pm \text{s.e. } 0.10 \mu\text{g cm}^{-2} \text{ min}^{-1}$) was significantly higher ($P < 0.02$) than in the control, non-pregnant group ($0.49 \pm \text{s.e. } 0.04 \mu\text{g cm}^{-2} \text{ min}^{-1}$). In the post-natal period their mean SER ($0.52 \pm \text{s.e. } 0.06 \mu\text{g cm}^{-2} \text{ min}^{-1}$) dropped significantly ($P < 0.02$) to the control, non-pregnant level. The mean SER in women who breast-fed increased during preg-

¹ Hicks, R. M., and Wakefield, J. St. J., *Chem. Biol. Interact.*, **5**, 139 (1972).

² Price, J. M., Oser, B. L., Biava, C. G., Vogin, E. E., Steinfeld, J. L., and Ley, H. L., *Science*, **167**, 1131 (1970).

³ Egeberg, R. O., Steinfeld, J. L., Frantz, I., Griffiths, G. C., Knowles, H., Rosenow, E., Sebrell, H., and Van Itallie, T., *J. Amer. Med. Assoc.*, **211**, 1358 (1970).

⁴ Epstein, S. S., Hollaender, A., Lederberg, J., Legator, M., Richardson, H., and Wolff, A. H., *Science*, **166**, 1575 (1969).

⁵ *Food Chemical News*, **12**, (2) 14 (1970).

nancy ($0.79 \pm \text{s.e. } 0.14 \mu\text{g cm}^{-2} \text{ min}^{-1}$) but the SER did not decrease during lactation. The mean SER during lactation ($0.87 \pm \text{s.e. } 0.13 \mu\text{g cm}^{-2} \text{ min}^{-1}$) was significantly higher ($P < 0.02$) than in both post-partum, non-lactating women and non-pregnant controls (Fig. 1).

SER increases during the last trimester of pregnancy⁷. This suggests that a powerful sebrotrophic hormone is produced during pregnancy, as the increase occurs when large quantities of oestrogens are being produced, and oestrogens are known to suppress sebum production. Our present data confirm this idea and suggest that suckling leads to further secretion of the sebrotrophic factor.

We now have evidence on the nature of this sebrotrophic factor from studies in both rat and man. In Parkinsonism the associated seborrhoea is induced by excessive secretion of a pituitary sebrotrophic hormone⁸⁻¹⁰. As restoration of mid-brain dopamine by L-dopa decreases the SER in Parkinsonism, this postulated sebrotrophic hormone must be under inhibitory control¹⁰. This excludes the known anterior pituitary sebrotrophic hormones¹¹⁻¹⁴. Prolactin and melanocyte-stimulating hormone (MSH) are the two pituitary hormones known to be under inhibitory control and prolactin is not sebrotrophic in the hypophysectomized or intact rat (ref. 15 and Thody and Shuster, unpublished results). In addition, administration of MSH stimulates sebum production in the rat¹⁷, and removal of the neuro-intermediate lobe—the site of secretion of MSH—leads to a profound decrease in sebum secretion which is restored by MSH^{16,17}. We have therefore proposed that this intermediate lobe sebrotrophic hormone is MSH or an MSH-like peptide¹⁷. This could explain the effect of pregnancy and lactation on sebum production, for MSH excretion increases during pregnancy^{18,19}, and in the rat suckling causes release of MSH from the pituitary^{20,21}. The same hormone may be responsible for the seborrhoea of Parkinsonism, and its release is presumably inhibited by L-dopa. L-Dopa may produce clinical remission of breast cancer by inhibiting prolactin release^{22,23}. In view of our present findings the alternative suggestion, that MSH may provide the endocrine drive in breast cancer, should be considered. If this is correct, MSH-inhibiting factor may be useful in the treatment of breast cancer.

J. L. BURTON
SAM SHUSTER
M. CARTLIDGE
L. J. LIBMAN
U. MARTELL

Wellcome Laboratories for Research into
Diseases of the Skin,
University Department of Dermatology,
Newcastle upon Tyne

Received December 18, 1972; revised January 15, 1973.

- ¹ Krant, M. J., Brandrup, C. S., Greene, R. S., Pochi, P. E., and Strauss, J. S., *Nature*, **217**, 463 (1968).
- ² Burton, J. L., Cunliffe, W. J., and Shuster, S., *Brit. Med. J.*, **1**, 665 (1970).
- ³ Wang, D. Y., Bulbrook, R. D., Guilleband, J., and Lewis, A., *Europ. J. Cancer*, **8**, 381 (1972).
- ⁴ Strauss, J. S., and Pochi, P. E., *J. Invest. Derm.*, **36**, 293 (1961).
- ⁵ Cunliffe, W. J., and Shuster, S., *Brit. J. Derm.*, **81**, 697 (1969).
- ⁶ Burton, J. L., Cunliffe, W. J., and Shuster, S., *Brit. J. Derm.*, **82**, 497 (1970).
- ⁷ Burton, J. L., Cunliffe, W. J., Millar, D. G., and Shuster, S., *Brit. Med. J.*, **2**, 769 (1970).
- ⁸ Burton, J. L., and Shuster, S., *Lancet*, **ii**, 19 (1970).
- ⁹ Burton, J. L., Cartledge, M., Cartledge, N. E. F., and Shuster, S., *Brit. J. Derm.* (in the press).
- ¹⁰ Burton, J. L., Cartledge, M., and Shuster, S., *Brit. J. Derm.* (in the press).
- ¹¹ Ebling, F. J., Ebling, E., and Skinner, J., *J. Endocrin.*, **45**, 245 (1969).
- ¹² Nikkari, T., and Valavaara, M., *J. Endocrin.*, **43**, 113 (1969).
- ¹³ Thody, A. J., and Shuster, S., *J. Endocrin.*, **47**, 219 (1970).
- ¹⁴ Thody, A. J., and Shuster, S., *J. Endocrin.*, **48**, 139 (1970).
- ¹⁵ Thody, A. J., and Shuster, S., *J. Endocrin.*, **51**, vi (1971).
- ¹⁶ Thody, A. J., and Shuster, S., *Nature*, **237**, 346 (1972).

- ¹⁷ Shuster, S., and Thody, A. J., *Advances in Biology of Skin—The Sebaceous Glands* (Pergamon Press, in the press).
- ¹⁸ Dahlberg, B. C. G., *Acta Endocrinologica*, **38**, Suppl. 60 (1961).
- ¹⁹ Shizume, K., and Lerner, A. B., *J. Clin. Endocrin.*, **14**, 1491 (1954).
- ²⁰ Taleisknik, S., and Orlas, R., *Endocrinology*, **78**, 522 (1966).
- ²¹ Taleisknik, S., and Tomatis, M. E., *Neuroendocrinology*, **3**, 303 (1968).
- ²² Stoll, B. A., *Lancet*, **i**, 431 (1972).
- ²³ Minton, J. P., and Dickey, R. P., *Lancet*, **i**, 1069 (1972).

The Effects of Cardioactive Drugs on the Performance of Cultured Foetal Hearts

A CLASSICAL method of organ culture has been established by the work of Dame Honor Fell. The techniques for maintaining mature mouse foetal hearts in organ culture were optimized by Wildenthal who found substances and mechanisms for prolonging their survival¹⁻³ and used them to study some cardioactive drugs⁴.

We wished to study the biological effects of implants of foetal heart, or extracts, in damaged areas of the heart. We were particularly interested in the first 20–30% of term when most human abortion material would be available. In the species we studied, we found that there was a direct relationship between the stage of development of foetal hearts and their survival in organ culture. The younger hearts lived longer than the more mature hearts⁵. A study of composite foetal hearts within and across the species barrier showed that fusion of the component parts and synchronous beating at the rate of the fastest component occurred sooner in less mature hearts⁶.

Foetal hearts are sensitive to chemical contamination of the culture medium, causing early death and diminished activity. Hearts cultured on pledgets of PVC containing ethylene oxide residues after sterilization, for example, died early and could be used to detect sterilization failures. Hearts cultured in chambers made of perspex tube with an odour thought to be uncured monomer died prematurely. We also found that hyperbaric oxygen is more toxic to young foetal hearts before innervation than it is to older hearts⁷.

Foetal hearts might therefore be used to determine the potential toxicity of compounds at various stages of foetal development. Also, the chronic effects of cardioactive drugs, such as β -blockers and catecholamines, might be different in hearts before, during and after innervation, giving insight into their mode of action. The beating rates of foetal hearts can be monitored simply and accurately, giving additional information to the crude survival times obtainable from non-active cultures. A substance that killed a culture prematurely, whatever its effect on the beating rate, was considered non-beneficial. If it increased the survival time without reducing the beating performance, the pure drug and its simple breakdown products were presumed to be non-toxic, at least to foetal heart tissue. Some substances increase survival time but depress activity, the total number of beats may be reduced, and such a substance is probably non-beneficial.

These studies could be done on mouse foetal heart tissue and checked in any other species, including man, in which the drug might be used as a therapeutic agent. As the human foetal heart is presently difficult to obtain and larger than the mouse heart, it is more difficult to culture and to assess its performance, and our preliminary study was done on mouse foetal hearts.

The strength and regularity of contraction and synchrony between the atria and ventricles varied between hearts cultured under the same conditions and from day to day in the same heart, and therefore we confined our observations to beating rates. The daily mean atrial beating rates for hearts in the nine different media are shown in Fig. 1 and the ventricular rates in Fig. 2. The curve for the control cultures is superimposed on each graph. Results are summarized in Table 1.

Table 1 Summary of Effects of Test Drugs

Drug ($\mu\text{g ml}^{-1}$)	Atria	Ventricles
Control	Exponential decay.	Exponential decay. Beating rate slower than atria and ceases sooner.
Tyramine 40	Overall depression of beating rate. Decrease in survival time.	Similar to controls.
Oxyfedrine 20	The curve swung to a more horizontal position. 0-8 d beating slowed. 9-19 d rate speeded up. Slight increase in survival time.	Curve swung to more horizontal position. Rate decreased up to day 4, rate increased from day 5-16. Survival time increased.
Oxyfedrine 40	Slight but uniform increase in rate from day 2 to day 15. No increase in survival time.	Very slight decrease in rate up to day 6, increase in rate from day 7 to 14. No increase in survival time.
Oxyfedrine } Tyramine } 40	Similar to control; the two drugs cancelling each other out.	Similar to control. Drugs cancel.
Oxyfedrine 80	Similar to control; striking reduction in the variability of the rate. (Scatter.)	Uniform slight increase in rate. Reduction in scatter as for atria.
Isoprenaline 50	Moderate overall increase in rate but no increase in survival time.	Moderate overall increase in rate; no increase in survival. Greater scatter.
L-Noradrenaline 15	Similar to oxyfedrine 20. Curve swung to horizontal position. Decreased rate 0-8 d, increased rate days 9-23. Survival time increased more than by oxyfedrine 20.	No significant effect from day 0-5; increased rate from day 6-17. Increase in survival time.
Practolol 50	0-8 d similar control, 9-18 d rate increased. Slight increase in survival time.	Overall increase in rate starting slight and becoming large. Large increase in survival time.

The four hearts under each set of conditions represent a range of foetal ages, and therefore a range of developmental stages, giving a certain amount of variation in beating performance.

The performance of the youngest and oldest groups of hearts were examined separately, and predictably the youngest hearts lived longer and beat better than the oldest. The atrial and ventricular beating rates of the oldest control hearts were erratic in this preliminary series, and we suggest that for drug evaluation, hearts of less than 60% of term give more meaningful results.

Some interesting differential effects were demonstrated. Oxyfedrine, for example, at $20 \mu\text{g ml}^{-1}$ showed a positive effect on both atria and ventricles in the young hearts with no apparent effect on the beating of the older atria and ventricles; whereas isoprenaline affected both the young and old hearts equally.

As well as being a potentially useful assay for cardioactive drugs, it seems also that any substance which is to be given to pregnant women should be examined for its direct effect on the mouse foetal heart. Further study with human foetal heart should follow if any effect is demonstrated. It cannot be assumed, however, that the substance is harmless if there is no effect on mouse or human foetal heart; many drugs are not pharmacologically active in their pure form, whereas their breakdown products are. Complete drug evaluation will require cultures with each of the intermediate breakdown products at their likely concentrations. If after this screening a drug seems to have no effect, or even a beneficial one, it would

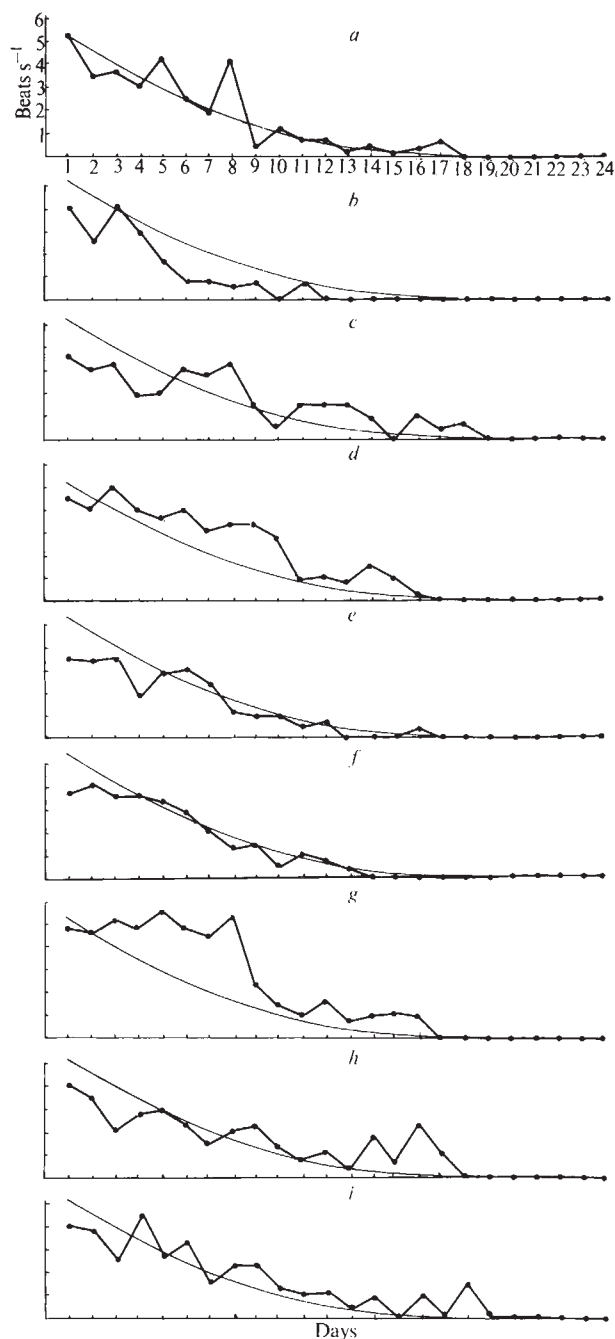


Fig. 1 Atrial rates of the control hearts compared with the atrial rates of the test hearts for the first 23 d of culture. Hearts from nine litter mates of comparable size were selected from pregnant mice for each set of experiments. (When one pregnant mouse contained only seven foetuses, two foetuses of the same crown rump size from another litter had to be used.) The experiment was repeated successfully four times, using a range of foetal ages 54% to 80% term (crown rump 9 to 16 mm). Hearts were cultured as previously described⁵ on stainless steel mesh so that their lower surfaces were wetted by 65% medium 199 (Burroughs Wellcome), with antibiotics, 35% foetal calf serum, $50 \mu\text{g ml}^{-1}$ insulin, $0.1 \mu\text{g ml}^{-1}$ cortisol; in a water vapour saturated atmosphere of 95% oxygen and 5% CO_2 and at a temperature of $37^\circ\text{C} \pm 2^\circ\text{C}$. Six drugs were added to this standard medium to give the nine test media, as follows: (a) standard medium (control); (b) medium + tyramine hydrochloride $40 \mu\text{g ml}^{-1}$; (c) medium + oxyfedrine $20 \mu\text{g ml}^{-1}$; (d) medium + oxyfedrine $40 \mu\text{g ml}^{-1}$; (e) medium + tyramine hydrochloride $40 \mu\text{g ml}^{-1}$ plus oxyfedrine $40 \mu\text{g ml}^{-1}$; (f) medium + oxyfedrine $80 \mu\text{g ml}^{-1}$; (g) medium + isoprenaline $50 \mu\text{g ml}^{-1}$; (h) medium + L-noradrenaline $15 \mu\text{g ml}^{-1}$; (i) medium + practolol $50 \mu\text{g ml}^{-1}$. The medium was replaced every other day after observations had been made. The cultures were examined daily using a dissecting microscope, during which examination special efforts were made to maintain the same constant conditions of temperature, atmosphere and water vapour saturation.

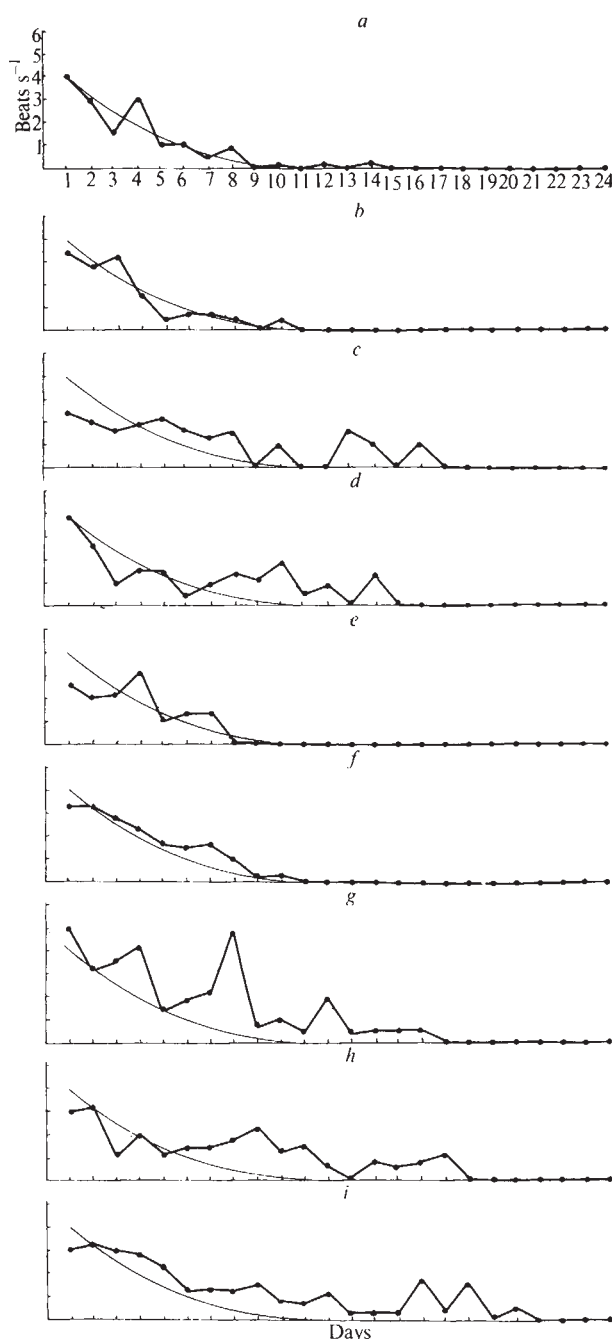


Fig. 2 Ventricular rates of the control hearts compared with the first 23 d for the test hearts.

seem reasonable to continue with further conventional clinical trials. If a new drug is depressant to the foetal myocardium above a certain concentration, it would be unwise to exceed this in a subsequent clinical trial. If the substance were to affect only young foetal hearts it might still be safe in late pregnancy.

We thank the Board of Governors of the National Heart and Chest Hospitals and the British Heart Foundation for financial support, Miss D. M. Hughes and our colleagues for help and Dame Honor Fell and Kern Wildenthal for encouragement.

S. R. ARMSTRONG
D. B. LONGMORE

National Heart Hospital,
Westmoreland Street,
London W1M 8BD

Received January 25; revised April 10, 1973.

- ¹ Wildenthal, K. J., *Proc. Physiol. Soc.*, **207**, 33 (1969).
- ² Wildenthal, K. J., *J. Appl. Physiol.*, **30**, 153 (1971).
- ³ Wildenthal, K. J., *Proc. Physiol. Soc.*, **217**, 56 (1971).
- ⁴ Wildenthal, K. J., *Amer. J. Physiol.*, **221**, 238 (1971).
- ⁵ Hughes, D. M., and Longmore, D. B., *Nature*, **235**, 334 (1972).
- ⁶ Longmore, D. B., and Hughes, D. M., *Nature*, **238**, 40 (1972).
- ⁷ Nagler, J., and Longmore, D. B., *Nature*, **242**, 197 (1973).

Penetration of Asbestos through the Digestive Tract of Rats

MESOTHELIOMAS of the pleura and peritoneum¹⁻³ have been linked with the inhalation of asbestos fibres. We have shown⁴ that asbestos fibres are present in drinking water, beer, wine and other beverages. Asbestos fibres can penetrate the mucosa of the stomach and intestine⁵ and Telischi and Rubenstone⁶ have found asbestos material in a gastric carcinoma. Godwin and Jagatic⁷, reporting on mesotheliomas of the pleura caused by asbestos, noted that asbestos particles were widely distributed in various tissues. They suggested that particles may migrate from the lung through the blood and lymphatic system to all parts of the body. In the light of these reports, we surmised that asbestos consumed orally can pass through the gut into the blood stream and accumulate in various tissues.

To determine if this is the case we injected a suspension of asbestos fibres into the stomachs of rats. The suspension was prepared by shaking 2.5 g of chrysotile asbestos (Johns Manville No. 7RFO2) in 500 ml of water in a graduated cylinder. The suspension was allowed to settle for 30 min and the top 250 ml, which contained about 9.4×10^9 (1 mg) fibres ml^{-1} , was drawn off. Most of these fibres are 0.2 μm –2.0 μm long (Fig. 1). To ensure that fibres passed through the digestive tract without rats inhaling any of them, the asbestos was introduced into the gastrointestinal tract by opening the abdomen under anaesthesia and injecting the fibres directly into the stomach. Two to 4 d later the rats were killed and blood and tissues analysed for asbestos. To ensure that any fibres on the rat hair could not contaminate the organs removed during surgery, the rats were first thoroughly vacuumed and washed with double distilled water and methanol. During the removal of tissues wet towels were placed over the animals and around the incisions. Uncontaminated blood was drawn from the orbital sinus using a capillary tube.

To avoid formation of excess ash, which interferes with examination of specimens under the electron microscope, the tissues were first solubilized with soluene (Packard Instrument Company); the fibres were then centrifuged down, washed with methanol and ashed as previously described⁴. The ash was taken up in 1 ml of distilled water which had been filtered through a 0.2 μm filter, and 5 μl of this mixture was dropped on hydrophilic carbon-coated electron microscope grids supported on a ring peg. The grids were dried in a closed glass container (to avoid air contamination) under a heat lamp and were examined at magnifications of 20,000 and 80,000 with an electron microscope. The presence of chrysotile asbestos fibres was verified by electron diffraction. They were counted and the numbers corrected for dilution to determine the number in the original 1 ml sample. Results of recovery of asbestos fibres from rats treated in this way are shown in Table 1.

Rats in group 1 were given 9.4×10^9 fibres (1 ml) and killed 2 d later; those in group 2 received 94×10^9 fibres and were killed 4 d later. No asbestos fibres were detected in the blood of non-injected rats (controls) but $4.65 \times 10^6 \text{ g}^{-1}$ (a statistically significant increase) were found in the first group.

This is the first time direct physical evidence for the presence of asbestos fibres in the blood has been obtained. Four days after treatment the amount of asbestos in the blood of rats in group 2 had dropped to $1.19 \times 10^6 \text{ g}^{-1}$ even though these rats received ten times as much asbestos. This indicates that blood

Table 1 Numbers of Asbestos Fibres Recovered from Treated and Non-Treated Rats

	Controls mean*		Group 1† mean*		Group 2‡ mean*	
Blood	0.00	—	4.65¶	1.02	1.19	0.77
Spleen	2.33	0.67§	4.11	0.49	3.45	1.20
Omentum	2.46	0.46	2.74	0.87	18.25	7.79
Heart	2.09	0.41	—	—	2.28	0.40
Brain	0.05	0.02	0.31	0.19	0.29**	0.11
Lungs	1.06	0.26	1.80	0.54	1.74	0.26

* Average of five rats in all cases except where noted. † Animals injected with 9.4×10^9 fibres and tissues examined 2 d later. ‡ Animals injected with 94×10^9 fibres and tissues examined 4 d later. § Standard error of the mean. || Four rats only. ¶ $P < 0.01$; ** $P < 0.05$.

(The figures in the table are fibres $\text{g}^{-1} \times 10^{-6}$.)

can clear itself of the fibres. Tissues such as the spleen, heart and lung also seem to have, to a lesser extent, the ability to clear themselves of the asbestos fibres, as the figures obtained from rats in group 2 indicate. They are lower than those for group 1 rats even though they received a larger dose, but the tissues had longer to clear themselves. Indeed, recent work in our laboratory with radioactive asbestos indicated that some tissues, such as the lungs, can reduce their fibre content by half in 2 d. This clearing action could possibly have quite an effect on the numbers of fibres found in the liver and kidney of the rats but unfortunately, because of the considerable amount of ash present in these organs even after solvane treatment, we could not examine these specimens under the electron microscope.

Another factor may contribute to the higher fibre count obtained for the organs of rats in group 1 compared with animals in group 2, namely, that there were four times as many fibres in the blood of rats in group 1. The residual blood in the tissues on removal from the animal would of course contribute to the total fibre count of a particular tissue.

This factor could have created the anomaly in the fibre counts in brains. In the brains of treated rats this was about six times higher than in the controls, and the increase in fibre count is statistically significant for rats in group 2 but not, oddly, for rats in group 1 even though there were more fibres in these animals. The greater number of fibres per gram in the blood of rats in group 1 could be responsible for the larger standard error in the counts of rats in group 1 and therefore did not allow the values obtained to be significant.

The brain seems to have little ability to clear itself of asbestos fibres, but the omentum has even less. This tissue, which

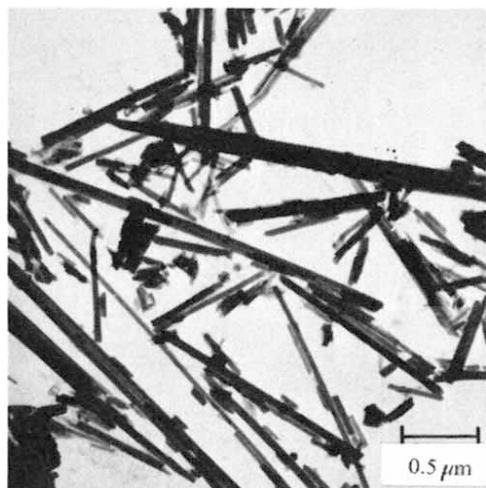


Fig. 1 Electron micrograph of the suspension of asbestos fibres injected into the rat stomach. Note the varying sizes of the bundles of fibres present.

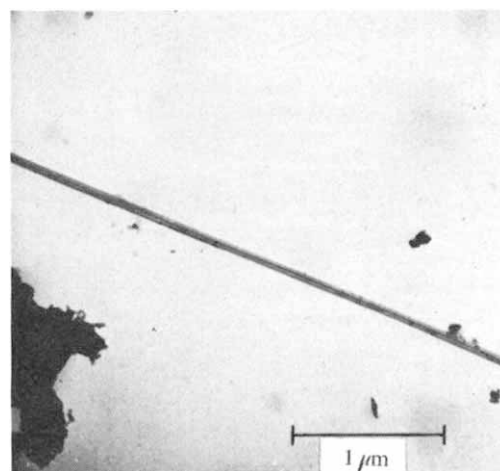


Fig. 2 Small bundle of asbestos fibres from the peritoneum of a rat. This bundle is at least 15 µm long.

surrounds the small intestine, appeared to accumulate most asbestos. Although the asbestos fibre content of the omentums in rats in group 2 may seem rather high (one extreme count contributed to the high standard error) the omentum does not seem to respond as do the other tissues. As well as having little ability to clear itself, fibre accumulation in the omentum may take place much more slowly than it does in other tissues. As the omentum consists partly of peritoneum and partly adipose tissue, asbestos fibres may enter the peritoneum by direct penetration of the intestinal wall. Concerning the prolonged retention of asbestos fibres in the omentum in women asbestos workers, Keal⁸ found a higher incidence of peritoneal cancer than of lung cancer.

The amount of asbestos found in the tissues of the controls is higher than we had anticipated, considering the age of the rats and the number of fibres they might have consumed in their drinking water⁴. But we do not know how much was in their feed or air. We have analysed the tissues of some people who died of natural causes and found somewhat similar levels. In one person there was twice as much asbestos in the brain as in the brains of our control rats and about one quarter as much in the omentum, but the omentum content was still higher than the brain.

Most of the fibres found were about 0.2–2.0 µm long but some measured 15 µm (Fig. 2) and one fibre in the blood of a rat was 23.55 µm long. We do not know how the fibres pass through the intestinal wall. Some long ones may pierce the gut like a needle, whereas pinocytosis may account for the absorption of the small ones.

We demonstrated earlier the presence of asbestos fibres in air, snow, city drinking water and a number of beverages⁴. The experiment described here shows that fibres of a similar size administered to the stomach of rats appear in the blood and accumulate in various tissues in the body.

R. D. PONTEFRACT
H. M. CUNNINGHAM

Food Research Laboratories,
Health Protection Branch,
Department of National Health and Welfare,
Ottawa

Received November 30, 1972; revised February 13, 1973.

- Hourihane, D. O. B., *Thorax*, **19**, 268 (1965).
- Hourihane, D. O. B., *Ann. NY Acad. Sci.*, **132**, 647 (1965).
- Lynch, K. M., and Smith, W. A., *Amer. J. Cancer*, **24**, 56 (1935).
- Cunningham, H. M., and Pontefract, R. D., *Nature*, **232**, 332 (1971).
- Selikoff, I. J., Churg, J., and Hammond, E. C., *J. Amer. Med. Assoc.*, **188**, 674 (1964).
- Telisch, M., and Rubenstone, A. I., *Arch. Pathol.*, **72** (1961).
- Godwin, M. C., and Jagatic, J., *Environ. Res.*, **3**, 391 (1970).
- Keal, E. E., *Lancet*, **ii**, 1211 (1960).

Presynaptic and Postsynaptic Effects of Lead at the Frog Neuromuscular Junction

LEAD has long been known to be toxic to animals and humans, with some of its effects being attributable to actions on the neuromuscular system^{1,2}. Since several other polyvalent cations have potent effects on neuromuscular transmission³⁻¹⁰, the question arose as to whether one of the manifestations of lead toxicity might be an interference with neuromuscular transmission. Answers to this question might elucidate some mechanisms of lead toxicity and, at the same time, improve our understanding of junctional transmission. This report shows that lead influences both pre and postsynaptic events in neuromuscular transmission with the presynaptic ones being most sensitive.

There have been no previous electrophysiological studies of the effects of lead on neuromuscular transmission. However, Kostial and Vouk²⁰, in a study of the perfused superior cervical ganglion of the cat, found that lead nitrate, in concentrations as low as 12.1 μM , blocked ganglionic transmission. The authors concluded that this effect was due to a presynaptic action of lead because: (a) the acetylcholine (ACh) output of the eserized ganglion, resulting from preganglionic nerve stimulation, was reduced by lead, and (b) the postganglionic response to perfused ACh was not affected by lead.

Experiments were performed *in vitro* on the isolated sciatic nerve-sartorius muscle preparation of the frog (*Rana pipiens*). Superficial neuromuscular junctions were located optically with a compound microscope (magnification $\times 400$). Standard microelectrode and photographic techniques were used to monitor intracellular responses from individual end-plates. Responses were recorded first while the preparation was bathed in a control Ringer solution, then in the presence of lead, added as PbCl_2 , and finally after the lead had been washed out. Solutions entered the experimental chamber by gravity and left by suction. The composition of the normal Ringer solution was: 111 mM NaCl, 2.5 mM KCl, 2.0 mM CaCl_2 , 4 mM Tris-maleate. All Ringer solutions were maintained at pH 6.9 and at a temperature of 15°C. Preparations were exposed to lead by bathing them in a Ringer solution to which 0.01–0.10 mM PbCl_2 had been added. To record subthreshold end-plate potentials, nerve-muscle preparations were bathed in 0.8 mM Ca^{2+} /5.5 mM Mg^{2+} -Ringer solution^{9,21}. Acetylcholine was applied directly to the end-plate receptors by iontophoresis from a micropipette^{22,23} in order to test for postsynaptic effects of lead.

We have consistently observed that lead increases the frequency of miniature end-plate potentials (Figs 1 and 2). The

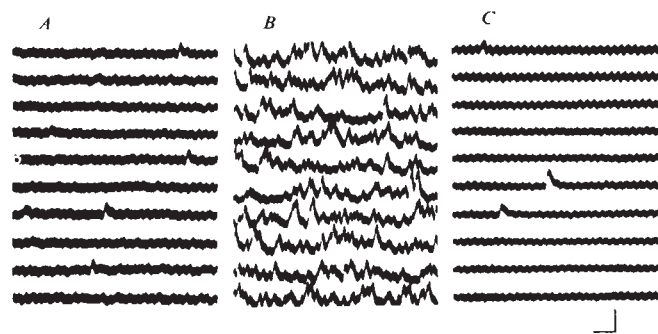


Fig. 1 Effect of lead on the frequency of miniature end-plate potentials. *A*, Control: the preparation was bathed in normal Ringer solution. *B*, Five minutes after exposing the preparation to 0.10 mM Pb^{2+} -Ringer solution: the frequency increased greatly while the amplitudes of the miniature end-plate potentials were essentially unchanged. *C*, Five minutes after washing the preparation with normal Ringer solution: the frequency was no longer elevated. Oscillograms *A–C* were taken from the same neuromuscular junction. Vertical calibration: 1 mV. Horizontal calibration: 50 ms.

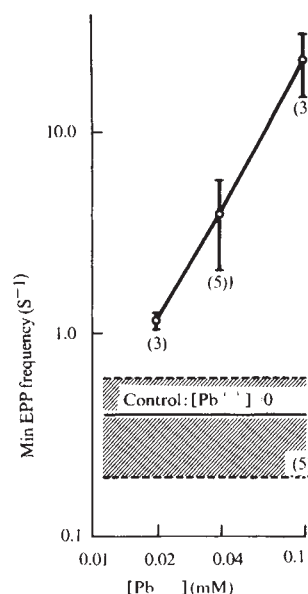


Fig. 2 A double logarithmic plot showing the dose-response relationship between the lead concentration and the frequency of miniature end-plate potentials. Each point is the mean $\pm$ s.e. for measurements made from three to five fibres. The control frequency was measured in the absence of lead and is depicted by horizontal lines (mean $\pm$ s.e.). All measurements were made after the preparations were exposed to the Pb^{2+} -Ringer solution for 5 min. Although not shown in the above plot, the mean frequency decreased to $0.42 \pm 0.10 \text{ s}^{-1}$ 5 min after washing the preparations with normal Ringer solution (see also Fig. 1C).

records shown in Fig. 1A were made while the preparation was bathed in normal Ringer solution; those in Fig. 1B were made after bathing the preparation in 0.10 mM Pb^{2+} -Ringer solution for 5 min; and those in Fig. 1C were recorded after washing with normal Ringer solution for 5 min. Data taken from several fibres are shown graphically in Fig. 2 which is a log-log plot of the relationship between the lead concentration and the frequency of miniature end-plate potentials. The frequency during control conditions was $0.39 \pm 0.20 \text{ s}^{-1}$ (mean $\pm$ s.e.), and it increased by almost two orders of magnitude in the presence of 0.10 mM Pb^{2+} , the actual value being $22.1 \pm 7.6 \text{ s}^{-1}$. When the fibres were washed with normal Ringer solution, the frequency returned to $0.42 \pm 0.10 \text{ s}^{-1}$ within 5–10 min. Thus, this effect of lead is reversible (see also Fig. 1C).

In 1954, del Castillo and Katz postulated that two modes of transmitter release exist at the frog neuromuscular junction—one for the spontaneous release of individual quanta and another for the synchronous or phasic release of several hundred quanta of the transmitter, ACh¹¹. This hypothesis is based on the observation that magnesium did not reduce the frequency of the spontaneous release of transmitter even though this ion had been shown previously to inhibit the phasic release following a nerve impulse⁹. Calcium antagonized this blocking effect of magnesium⁹, but it had no clear effect on the frequency of miniature end-plate potentials²⁴. Our experiments show that lead depresses the phasic release of transmitter (Fig. 3). Subthreshold end-plate potentials were recorded in 0.8 mM Ca^{2+} /5.5 mM Mg^{2+} -Ringer solution. The motor nerve was stimulated with pairs of pulses to facilitate transmitter release during the second transmission, thereby producing easily detectable (facilitated) end-plate potentials. The interval between pulses in a pair was 5–7 ms, and the pair of stimuli were applied at a rate of 3/s. Since the amplitude of end-plate potentials recorded from preparations bathed in high magnesium and low calcium Ringer solution varies according to the quantal hypothesis²⁵, many recordings were made in a particular condition to obtain the mean $\pm$ s.e. of the end-plate potential. The facilitated end-plate potential during the control period was $4.71 \pm 0.15 \text{ mV}$ ($n=18$) (Fig. 3A). The preparation was then exposed to 0.01 mM Pb^{2+} , the $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio

remaining unchanged; within 1 min, the facilitated end-plate potential decreased to 0.49 ± 0.08 mV ($n=21$, three failures). Figure 3B shows a typical effect of 0.01 mM Pb^{2+} —the facilitated end-plate potential was reduced to a single quantal response while the unfacilitated one was blocked completely. As 0.10 mM Pb^{2+} does not block miniature end-plate potentials (Fig. 1B), the blocking effect on the end-plate potential occurred presynaptically by decreasing the quantal release of acetylcholine. When the nerve-muscle preparation was washed with the control Ringer solution, the facilitated end-plate potential recovered almost completely within 10 min to 3.97 ± 0.10 mV ($n=38$) (Fig. 3C). Thus, this effect of lead is also reversible.

It is important to note that the subthreshold end-plate potential was reduced by 0.01 mM Pb^{2+} within less than 1 min whereas no significant increases in the frequency of miniature end-plate potentials were detected when this amount of lead was added to normal Ringer solution. Larger concentrations of lead were necessary to cause detectable increases in the frequency of the spontaneous release of transmitter (Figs 1 and 2). This dose differential, together with the two different effects of lead on transmitter release—stimulating its spontaneous release while inhibiting its phasic release—clearly indicate that these two processes are distinct. However, these processes may not be entirely independent²⁶.

To confirm our conclusion that lead reduced the end-plate potential by a presynaptic block and not by a postsynaptic one, other experiments were performed to see if lead decreased the ACh sensitivity of the postsynaptic (end-plate) membrane; this sensitivity was determined by dividing the magnitude of the ACh potential in mV by the total charge in nC that was delivered from the iontophoretic pipette²⁷. The ACh sensitivity was not affected by 0.01 mM Pb^{2+} ; however, 0.10 mM Pb^{2+} , a dose ten times that sufficient to reduce the end-plate potential in the experiments described above, was shown to have a weak curariform action (Fig. 4). Lead caused a small reduction (5.4%) in the ACh potential, which is the depolarization of the end-plate membrane following the iontophoretic application of ACh, when the tip of the ACh micropipette was very close to the receptors (Fig. 4A and B). In these conditions, the ACh sensitivity both in the absence and in the presence of lead was near 100 mV nC⁻¹ (ref. 28) and the density of ACh-receptor complexes probably approached that which occurs following the neural release of ACh. When this density was decreased by moving the ACh micropipette away from the receptors, however, the ACh sensitivity was 5.3 mV nC⁻¹ and fell to 0.9 mV nC⁻¹ (65% reduction) in the presence of lead. Thus, lead can only block postsynaptically in conditions in which the density of ACh-receptor complexes is much lower than it is during an end-plate potential.

The action of lanthanum at the frog neuromuscular

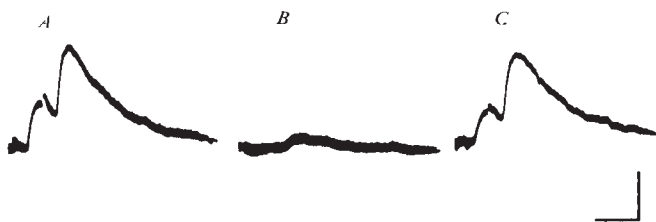


Fig. 3 Effect of lead on the end-plate potential. Neuromuscular transmission was blocked by bathing the nerve-muscle preparation in 0.8 mM Ca^{2+} / 5.5 mM Mg^{2+} -Ringer solution, thereby making it possible to record subthreshold end-plate potentials. Facilitated end-plate potentials were recorded intracellularly by stimulating the motor nerve with double shocks at a rate of 3 s⁻¹. *A*, Responses to nerve stimulation while the preparation was bathed in the control Ringer solution. *B*, Effect of a 1 min exposure to 0.01 mM Pb^{2+} added to the modified Ringer solution. Notice that 0.01 mM Pb^{2+} blocked the unfacilitated end-plate potential and reduced the facilitated one. *C*, Responses 10 min after the preparation was washed with the control Ringer solution. The amplitudes of the facilitated end-plate potentials in *A*–*C* are similar to the mean values which are given in the text. Vertical calibration: 2 mV. Horizontal calibration: 10 ms.

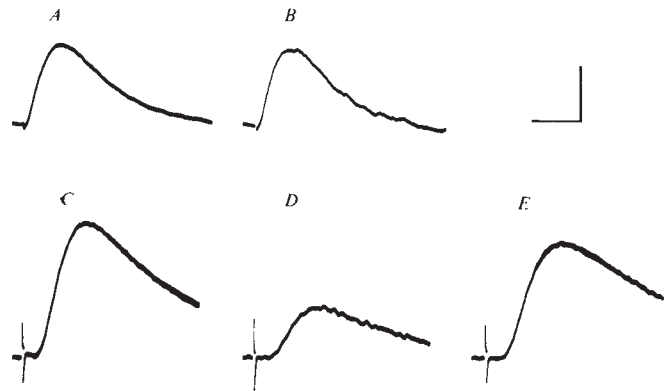


Fig. 4 Effect of lead on the amplitude of the ACh potential. *A*, ACh potential recorded from a neuromuscular junction bathed in normal Ringer solution. The tip of the ACh micropipette was optimally positioned at the end-plate receptors since the latency was very small (less than 1 ms) and the ACh sensitivity high (near 100 mV nC⁻¹). *B*, ACh potential recorded within 1 min after washing the preparation with 0.10 mM Pb^{2+} -Ringer solution; the iontophoretic current pulse and the position of the intracellular microelectrode and of the ACh micropipette were unchanged from *A*. *C*, ACh potential recorded several minutes later when the preparation was again bathed in normal Ringer solution. The ACh micropipette was moved away from the muscle fibre until a latency of 40 ms resulted. The ACh sensitivity was 5.3 mV nC⁻¹. *D*, ACh potential recorded within one minute after washing the preparation with 0.10 mM Pb^{2+} -Ringer solution. *E*, ACh potential recorded after washing the preparation with normal Ringer solution. The total charge delivered from the ACh micropipette and the position of the intracellular microelectrode and of the ACh micropipette were the same in *C*–*E*. Note that 0.10 mM Pb^{2+} significantly lowered the ACh potential in *D* whereas it lowered only very slightly the one in *B*. *A*–*E* were taken from the same end-plate. Records of the iontophoretic current are not present; only the iontophoretic artefacts appear on the voltage traces. The resting potential was -98 mV. Vertical calibration: 5 mV. Horizontal calibration: 20 ms in *A*–*B* and 100 ms in *C*–*E*.

junction^{9,13–17} is quite similar to the presynaptic effects of lead described in this report. Moreover, there are other ions, in addition to those of lead, lanthanum, and magnesium, which inhibit the phasic release of transmitter—namely, those of beryllium, manganese, nickel and zinc^{3,9,13–19}. It is not known precisely with which ligands of the presynaptic membrane these heavy metals combine. Perhaps we shall eventually be able to relate a specific physico-chemical characteristic of these metal ions to their potency as blockers of presynaptic release of transmitter. If so, we might thereby gain some insight concerning the chemical nature of those ligands that are involved in excitation-secretion coupling.

We thank Dr L. Michael for performing lead analyses and Dr D. Faber for criticizing our manuscript. This work was supported by a grant from the US National Institutes of Health.

R. S. MANALIS

Department of Physiology,
College of Medicine,
University of Cincinnati,
Cincinnati, Ohio 45219

G. P. COOPER

Departments of Environmental Health and Physiology,
College of Medicine,
University of Cincinnati,
Cincinnati, Ohio 45219

Received December 16, 1972; revised February 12, 1973.

- Cummings, J. N., *Heavy Metals and the Brain*, 95 (Thomas, Springfield, 1959).
- Lead: *Airborne Lead in Perspective*, 84, 178 (National Academy of Sciences, Washington DC, 1972).
- del Castillo, J., and Engbaek, L., *J. Physiol.*, **124**, 370 (1954).
- Jenkinson, D. H., *J. Physiol.*, **138**, 434 (1957).
- Dodge, jun., F. A., and Rahamimoff, R., *J. Physiol.*, **193**, 419 (1967).
- Katz, B., *The Release of Neural Transmitter Substances* (Thomas, Springfield, Illinois, 1969).

- ⁷ Meiri, U., and Rahamimoff, R., *J. Physiol.*, **215**, 709 (1971).
⁸ Heuser, J., Katz, B., and Miledi, R., *Proc. Roy. Soc.*, **B178**, 407 (1971).
⁹ Blioch, Z. L., Glagoleva, I. M., Liberman, E. A., and Nenashev, V. A., *J. Physiol.*, **199**, 11 (1968).
¹⁰ Hubbard, J. I., *J. Physiol.*, **159**, 507 (1961).
¹¹ del Castillo, J., and Katz, B., *J. Physiol.*, **124**, 553 (1954).
¹² Mambri, J., and Benoit, P. R., *CR Acad. Sci. Paris*, **158**, 1454 (1964).
¹³ Lambert, D. H., and Parsons, R. L., *J. Gen. Physiol.*, **56**, 309 (1970).
¹⁴ DeBassio, W. A., Schnitzler, R. M., and Parsons, R. L., *J. Neurobiol.*, **2**, 263 (1971).
¹⁵ Heuser, J., and Miledi, R., *Proc. Roy. Soc.*, **B179**, 247 (1971).
¹⁶ Kajimoto, N., and Kirpekar, S. M., *Nature New Biology*, **235**, 29 (1972).
¹⁷ Miledi, R., *Nature*, **212**, 1233 (1966).
¹⁸ Benoit, P. R., and Mambri, J., *J. Physiol.*, **210**, 681 (1970).
¹⁹ Meiri, U., and Rahamimoff, R., *Science*, **176**, 308 (1972).
²⁰ Kostial, K., and Vouk, V. B., *Brit. J. Pharmacol.*, **12**, 219 (1957).
²¹ Katz, B., and Miledi, R., *Proc. Roy. Soc.*, **B161**, 453 (1965).
²² Nastuk, W. L., *Fed. Proc.*, **10**, 96 (1951).
²³ del Castillo, J., and Katz, B., *J. Physiol.*, **128**, 157 (1955).
²⁴ Fatt, P., and Katz, B., *J. Physiol.*, **117**, 109 (1952).
²⁵ del Castillo, J., and Katz, B., *J. Physiol.*, **124**, 560 (1954).
²⁶ Quastel, D. M. J., Hackett, J. T., and Cooke, J. D., *Science*, **172**, 1034 (1971).
²⁷ Miledi, R., *J. Physiol.*, **151**, 1 (1960).
²⁸ Feltz, A., and Mallart, A., *J. Physiol.*, **218**, 85 (1971).

T Cell-dependent Mediator in the Immune Response

SUPERNATANTS of allogeneic lymphocyte cultures enable bone-marrow derived (lymphocytes) to respond to sheep red blood cells (SRBC) *in vitro*¹⁻³. It was suggested that a non-specific T cell-dependent mediator may be involved in the normal immune response to this antigen. Indeed, T cells educated to one erythrocyte antigen can, in the presence of that antigen, greatly facilitate the response of B cells to other non-cross reacting erythrocytes^{4,5}. T cells educated to protein antigens can also generate such a non-specific event in the interaction of T and B cells *in vitro*⁶. The following experiments were designed to establish whether in this syngeneic system the phenomenon was dependent on the production of soluble mediators after activation of T cells by protein antigen.

Mice of the CBA/Ca strain were used and were of the same sex in each experiment. As a source of B cells for culture we used the spleens of mice which had been thymectomized at 6 to 10 weeks of age, later irradiated and reconstituted with 4×10^6 syngeneic bone-marrow cells⁶. Educated T cells were obtained by using spleen cells of irradiated adult mice injected with 10^8 syngeneic thymocytes and 500 μ g of keyhole limpet haemocyanin (KLH) (Calbiochem.) without added adjuvant. B cells alone respond poorly to SRBC *in vitro* and the facilitation of this response was used as an assay for T cell activity, using T cells educated to KLH. This assay system is designed to detect non-specific facilitation.

Three types of culture conditions were adopted. (a) Double chamber cultures based on the technique of Marbrooke⁶ as modified by Feldmann and Basten⁷ were used in the first experiments. They differed only in that culture chambers were smaller and were contained within 20 ml screw cap bottles. B cells (10^7) were cultured in the outer chamber which was bathed in 8 ml of culture medium. Educated T cells (5×10^6) were added to the inner chamber as required. The two chambers were separated by a 0.4 μ m pore size 'Nuclepore' membrane. (b) For the 'macro-assay' of supernatant material a culture system based on that described by Mishell and Dutton⁸ was used, with the addition of nucleosides and mercaptoethanol as suggested by Click *et al.*⁹. B cells ($6-10 \times 10^6$) were cultured in 1 ml of medium. (c) To satisfy the need for a rapid and economical system to assay small amounts of supernatant, 6×10^5 B cells were cultured in Cooke's 'Microtiter' trays in a final volume of

Table 1 Geometric Means ($\pm$ s.d.) of the Plaque-forming Cell (PFC) Response to SRBC of Triplicate Cultures in a Modified Marbrooke Culture System

Inner chamber Antigen Cells	Outer chamber Antigen	Cells	PFC/culture (s.d.)
(1) KLH None	KLH and SRBC	$T_{KLH} + B$ cells	196 (338-112)
(2) KLH T_{KLH}	KLH and SRBC	B cells	207 (236-186)
(3) KLH None	KLH and SRBC	B cells	38 (70-22)
(4) None None	SRBC	T_{KLH} B cells	43 (80-24)

The system contained 1×10^7 B cells in the outer chamber and 5×10^6 T_{KLH} cells in the inner or outer chamber. KLH was added where indicated at a concentration of $30 \mu\text{g ml}^{-1}$ and 5×10^6 SRBC were added to the outer chamber of all cultures. T_{KLH} , T cells educated to KLH (see text).

100 μ l of medium (Dutton, personal communication). T cell supernatants were prepared by incubating 10^7 KLH-educated T cells with 30 μ g of KLH for 36 h in a 1 ml culture fluid volume. The supernatant was then collected, and the cells spun down. In the three culture systems the plaque-forming cell (PFC) responses to SRBC were assayed after 4 d in culture.

Table 1 shows the result of an experiment in which KLH-educated T cells were cultured either together with (line 1) or separated from B cells by a 'Nuclepore' membrane (line 2). Facilitation of the B cell response occurred and was similar even when T cells were separated from B cells by the 'Nuclepore' membrane. Neither KLH alone nor KLH-educated T cells alone could produce this effect (lines 3 and 4). The supernatants from educated T cells cultured with KLH were subsequently tested on B cells in Petri dish cultures as described above (Table 2). It was found that a stable factor capable of generating non-specific facilitatory activity had been produced. Unprimed, passaged T cells could not generate such activity when cultured in the same way. These observations were also true in the microculture system, and Fig. 1 shows that the supernatant activity in such a system could be titrated. A 'micro-assay' system of this nature makes it possible to attempt chemical characterization of the material. This supernatant activity is non-dialysable and can withstand freezing to -20°C . It is also possible to concentrate the activity 10-fold (by an Amicon Diaflow cell). We are now further characterizing this concentrated material.

The supernatant does not act by recruiting T cells specific for SRBC which may contaminate the B cells. Treatment of B cells before culture with anti- θ and guinea-pig complement¹⁰ to remove any contaminating T cells did not prevent the B cell response to SRBC in the presence of the supernatant of educated T cells.

Preliminary experiments designed to study the events involved in the production of this factor during the incubation *in vitro* showed that the addition of adherent cells to the cultures of

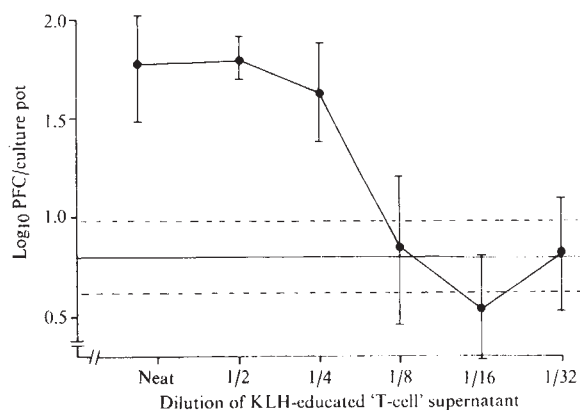


Fig. 1 Titration of the activity of T cell culture supernatant on the PFC responses of 6×10^5 B cells in microcultures. Each point represents the geometric mean of six replicate pots (with s.d.). Also shown is the effect of control medium (with s.d.).

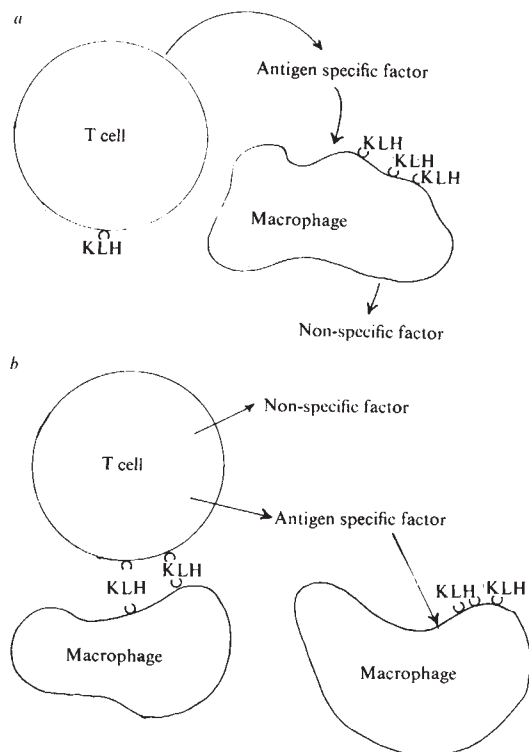


Fig. 2 Two possible mechanisms (a and b) for the involvement of adherent cells in the production of specific and non-specific factors by educated T cells.

educated T cells greatly enhanced the activity of the resulting supernatant (Table 3). In these experiments the adherent cells were prepared from B mice. It seems that, in the spleen cells from lethally irradiated mice reconstituted with thymocytes, there are insufficient functional macrophages to produce an optimal effect. It remains to be established whether the chemical nature of the active supernatant factor made with limiting numbers of adherent cells is identical to the supernatant factor made with excess adherent cells.

Table 2 Geometric Means ($\pm$ s.d.) of the PFC Responses of Triplicate Cultures of 6×10^6 Cells to SRBC in the Presence of T Cell Supernatants

Treatment	PFC/culture	Standard deviation
Supernatant from KLH-educated T cells incubated with KLH	765	738
Supernatant from "nil"-educated T cells incubated with KLH	94	79
Culture medium incubated for 36 h with KLH	67	47
2×10^6 KLH-educated T cells added directly to culture with KLH	2,822	2,344
		3,388

"Nil"-educated T cells are taken from spleen of irradiated adult mice injected with 1×10^8 thymocytes and no antigen.

Table 3 Geometric Mean of the PFC Responses of Twelve Replicate Microcultures of 6×10^5 B Cells to SRBC in the Presence of "Supernatants" Prepared in Various Ways

Preparation of supernatant	PFC/culture pot	Expt 1	Expt 2
Medium incubated with KLH	4	1	
Educated T cells with KLH	16	25	
Adherent cells with KLH	7	7	
Adherent cells with educated T cells and KLH	95	90	

Adherent cells required for the preparation of supernatants were obtained by incubating 6×10^6 spleen cells from B mice on Falcon Petri dishes, and after washing the monolayer thoroughly with medium for 1 h. Each culture contained 50 μ l of the appropriate supernatant, 50 μ l of B cells and 10 μ l of SRBC containing 10^8 cells ml^{-1} .

In conclusion, our data demonstrate the generation of a supernatant factor by educated T cells in the presence of specific antigen, which can facilitate the response of B cells to a non-cross reacting erythrocyte antigen. We are unable to state whether the non-specific component of this phenomenon comes directly from T cells, or from other cell types or serum factors in the culture. The dramatic effect produced by the addition of adherent cells to T cell cultures suggests, however, that macrophages are intimately involved in its production, either directly or indirectly. Feldmann¹¹ has demonstrated that T cells produce a factor with immunological specificity which, when bound to macrophages and complexed with antigen, can induce B cells to respond specifically. Possible mechanisms for the involvement of adherent cells in the production of non-specific factor are (Fig. 2): (a) The production by KLH-stimulated T cells of a molecule of the type described by Feldmann which, when complexed with specific antigen, might activate macrophages to produce the non-specific factor. Indeed macrophages may release non-specific mitogenic substances when cultured in the presence of con A or endotoxin¹². (b) Macrophages may be required to present antigen (KLH) to T cells for their optimal stimulation and subsequent production of non-specific and specific factors. These two models are not mutually exclusive.

If, however, macrophages are responsible for the production of non-specific factor, then the possibility must be considered, either that non-specific factor can provide the decisive signal directing a cell to induction, or that it may have a facilitatory role in lowering the threshold of triggering by latticed antigen. Such a factor may be an important component of adjuvant effect and may in part account for many experimental observations in which antigenic promotion or apparent lack of carrier specificity have been demonstrated¹³⁻¹⁵.

We thank Mrs K. Day and Miss Y. Pettit for their help, Mr B. W. Gurner and Mrs J. Waldmann for the figures, and Professor R. R. A. Coombs for encouragement. The work was supported by grants from the Medical Research Council.

H. WALDMANN
A. MUNRO

Immunology Division, Department of Pathology,
University of Cambridge, Tennis Court Road, Cambridge

Received December 11, 1972.

- Schimpl, A., and Wecker, E., *Nature New Biology*, **237**, 15 (1972).
- Feldmann, M., and Basten, A., *J. Exp. Med.*, **136**, 722 (1972).
- Hartmann, K. U., *J. Exp. Med.*, **133**, 1325 (1971).
- Hunter, P., Munro, A., and McConnell, I., *Nature*, **236**, 52 (1972).
- Waldmann, H., Munro, A., and Hunter, P., *Europ. J. Immunol.*, **3**, 167 (1973).
- Marbrooke, J., *Lancet*, ii, 1279 (1967).
- Feldmann, M., and Basten, A., *Nature New Biology*, **237**, 12 (1972).
- Mishell, R. I., and Dutton, R. W., *J. Exp. Med.*, **126**, 423 (1967).
- Click, R. E., Benck, L., and Alter, B. J., *Cell. Immunol.*, **3**, 264 (1972).
- Golub, E. S., *Cell. Immunol.*, **2**, 353 (1971).
- Feldmann, M., *J. Exp. Med.*, **136**, 757 (1972).
- Gery, I., and Waksman, B. H., *J. Exp. Med.*, **136**, 143 (1972).
- Katz, D. H., Paul, W. E., Goidl, E. A., and Benacerraf, B., *J. Exp. Med.*, **133**, 169 (1971).
- Osborne, jun., D. P., and Katz, D. H., *J. Exp. Med.*, **136**, 439 (1972).
- Ordal, J. C., and Grumet, F. C., *J. Exp. Med.*, **136**, 1195 (1972).

Depression of Freezing Point by Glycoproteins from an Antarctic Fish

SCHOLANDER and colleagues¹⁻³ observed that the blood sera of some polar fishes contain a substance of high molecular weight which lowers the freezing point. The general properties and structures of a family of several glycoproteins with this characteristic have recently been described in Antarctic fishes⁴⁻¹⁴. These "antifreeze" glycoproteins

(AFGP) consist of repeating units of the triglycopeptide Ala-Ala-Thr-*o*-disaccharide. Three active glycoproteins have been characterized; these differ only in polymer length, with molecular weights ranging from 10,500 to 21,000 g mol⁻¹ as determined by ultracentrifugation, light scattering, or osmotic pressure^{6,11}. According to the freezing point depression, however, the apparent molecular weight is only 20 g mol⁻¹, a value equivalent to >500 times the depression calculated from the molecular weights.

The blood sera of two species of Antarctic fish, *Trematomus borchgrevinki* and *Dissostichus mawsoni*, freeze at approximately -2.0° C, slightly below the temperature of the ice-salt water mixture in Antarctica⁵. Approximately one-third of the depression of the freezing point is caused by the AFGP, the remainder by dialysable substances of low molecular weight⁵. We have examined the rates of development of ice crystals and possible equilibria between ice crystals and aqueous phases, using differential thermal analysis (DTA) and direct microscopic observations of freezing and melting, respectively. The AFGP was purified from *T. borchgrevinki* serum as described previously^{6,10}.

The DTA experiments were carried out in aluminium vessels in a Mettler vacuum thermal analyser. Controls of water or solutions of 1% chicken ovomucoid¹⁵ (a glycoprotein containing approximately 25% carbohydrate) in water and the solutions containing 1% AFGP froze at the same temperatures and had similar freezing and melting curves. Freezing of small volumes (10 λ) of such solutions usually occurred between -15 and -20° C, depending on the purity of the water and the experimental rates of lowering the temperature. The freezing temperatures in these conditions are usually considered to be related to the rates of nucleation and not to their freezing temperatures in equilibrium with ice. These experiments did not, therefore, indicate that the AFGP functions by inhibiting nucleation, that is, by inhibiting the initial formation of points of crystallization.

Direct observations of melting and freezing in volumes of approximately 50 λ at closely controlled temperatures were made with a Zeiss Universal polarization microscope (magnification 90) equipped with a photographic camera and a television screen monitor. The microscope slides were double-celled, with a compartment on each side of a centre divider. The whole slide was cooled by a slow flow of cold nitrogen gas and the temperature was maintained by heat supplied to the centre of the slide by a heating element in the divider. The thermocouple was Pt-PtRh, positioned in the centre of the slide. The apparatus was calibrated by determining the melting and freezing temperatures of pure water. A series of eight experiments was done and each experiment had five to twenty separate melting and thawing trials. Results of a typical experiment are summarized in Table 1.

In one series of trials, solutions of egg white ovomucoid were used in the control well of the microscope slide. The AFGP and the ovomucoid were both tested as 1% solutions in water. In all trials, both the melting and freezing point of the ovomucoid solution were $-0.02 \pm 0.01^\circ \text{C}$. The melting point of an AFGP frozen solution or of ice crystals in AFGP solution was -0.01°C , and the freezing point of the solution containing ice crystals was -0.80°C . In another series of trials, a solution of AFGP containing a few crystals of ice froze at -0.78°C and melted at 0.00°C . After freezing, the sample was melted at $+1^\circ \text{C}$ until only a small bundle of crystals remained (approximately 0.1% of the solution had crystals). The sample was then adjusted to -0.60°C and held at this temperature for 300 min. No growth or melting of the crystals occurred. Similar observations were made when the FPDG solutions containing ice crystals were maintained at a temperature slightly less than the melting point (-0.10°C) or slightly more than the freezing point (-0.70°C).

From our experiments we conclude that: (1) Ice formed in a solution of AFGP seems to be normal ice—that is, it

Table 1 Freezing and Melting of Water and Solution of Antifreeze Glycoproteins in Water

Temperature adjustments	In water containing ice crystals	Observed changes In water solution of 1% antifreeze glycoproteins containing ice crystals
0°C phase	Melt and freeze	Crystals melt
-0.1°C lowering	Frozen	Crystals do not melt, liquid does not freeze
-0.7°C lowering	Frozen	Crystals grow, new crystals form until all solution frozen
-0.8°C holding	Frozen	All frozen, no melting
-0.7°C raising	Frozen	All frozen, no melting
-0.1°C raising	Frozen	All frozen, no melting
0.0°C holding	Melt and freeze	Melt

Water and antifreeze glycoprotein solution was initially frozen at -3°C and then allowed to melt at $+0.1^\circ \text{C}$ until 5–10% of solution remained as ice crystals. The temperature was then adjusted to 0.0°C and periodically lowered and then raised as indicated. The times at each temperature intermediate between freezing and melting were 5–10 min. All observations were made microscopically as described in the text.

melts at 0°C . (2) Freezing and melting of AFGP solutions occur at rates similar to those at which water freezes and melts when equivalent amounts of heat are applied or removed at the respective melting or freezing temperatures. Thus there was no evidence indicating a comparatively rapid development of crystals in AFGP solutions as described by Hargens¹⁶. (3) It is not possible to prove a mechanism involving nucleation from the DTA experiments on kinetic effects. There are, therefore, no unusual "supercooling effects" as are commonly found in solutions in which initiation of freezing is very slow in the absence of crystals. The data indicate that there is no significant kinetic effect involved in the overall freezing mechanism.

The pronounced hysteresis and absence of equilibrium between the melting and freezing of solutions of AFGP are consistent with a mechanism which is not based on colligative properties. Models for the mechanism could include those postulating effects on either the structure or growth of ice crystals or the structure of water. If a model concerning ice is correct, the mechanism would most likely involve the development of ice crystals^{10,17} after nucleation. If the model concerning the structure of water is correct, the mechanism could involve either the structuring of water itself or some intermediate state. The fact that ice crystals in AFGP solutions have normal melting points lends credence to the latter model.

We thank Dr Hansa Ahrends for supervising the DTA experiments and for his advice and suggestions, the Mettler Corp. for assistance, and Dr Richard Criddle for discussions and suggestions regarding the mechanism of action. The investigation was supported by funds from the Eidgenössische Technische Hochschule. R. E. F. was on sabbatical leave from the University of California, Davis.

R. E. FEENEY*
R. HOFMANN

Laboratorium für Festkörperphysik,
Eidgenössische Technische Hochschule,
Zürich, Hönggerberg, CH-8049 Zürich

Received January 23, 1972.

* Permanent address: Department of Food Science and Technology, University of California, Davis, California 95616.

¹ Scholander, P. F., Flagg, W., Walters, V., and Irving, L., *Physiol. Zool.*, **26**, 67 (1953).

² Scholander, P. F., van Dam, L., Kanwisher, J. W., Hammel, H. T., and Gordon, M. S., *J. Cell Comp. Physiol.*, **49**, 5 (1957).

³ Gordon, M. S., Amdur, B. H., and Scholander, P. F., *Biol. Bull.*, **122**, 52 (1962).

⁴ De Vries, A. L., and Wohlschlag, D. E., *Science*, **163**, 1073 (1969).

⁵ Komatsu, S. K., thesis, Univ. California, Davis (1969).

⁶ De Vries, A. L., Komatsu, S. K., and Feeney, R. E., *J. Biol. Chem.*, **245**, 2901 (1970).

- ⁷ Komatsu, S. K., DeVries, A. L., and Feeney, R. E., *J. Biol. Chem.*, **245**, 2909 (1970).
- ⁸ DeVries, A. L., Vandenheede, J., and Feeney, R. E., *J. Biol. Chem.*, **246**, 305 (1971).
- ⁹ Shier, W. T., Lin, Y., and DeVries, A. L., *Biochem. Biophys. Acta*, **263**, 406 (1972).
- ¹⁰ Vandenheede, J. R., Ahmed, A. I., and Feeney, R. E., *J. Biol. Chem.*, **247**, 7885 (1972).
- ¹¹ Feeney, R. E., Vandenheede, J., and Osuga, D. T., *Naturwissenschaften*, **59**, 22 (1972).
- ¹² DeVries, A. L., in *Fish Physiology* (edit. by Hoar and Randall), **6**, 157 (Academic Press, New York, 1969).
- ¹³ Chuba, J. V., Kuhns, W. J., Nigrelli, R. F., Vandenheede, J., Osuga, D. T., and Feeney, R. E., *Nature*, **242**, 342 (1973).
- ¹⁴ DeVries, A. L., *Science*, **172**, 1152 (1971).
- ¹⁵ Feeney, R. E., and Allison, R. G., *Evolutionary Biochemistry of Proteins* (Wiley-Interscience, New York, 1969).
- ¹⁶ Hargens, A. R., *Science*, **176**, 184 (1972).
- ¹⁷ Scholander, P. F., and Maggert, J. E., *Cryobiology*, **8**, 371 (1971).

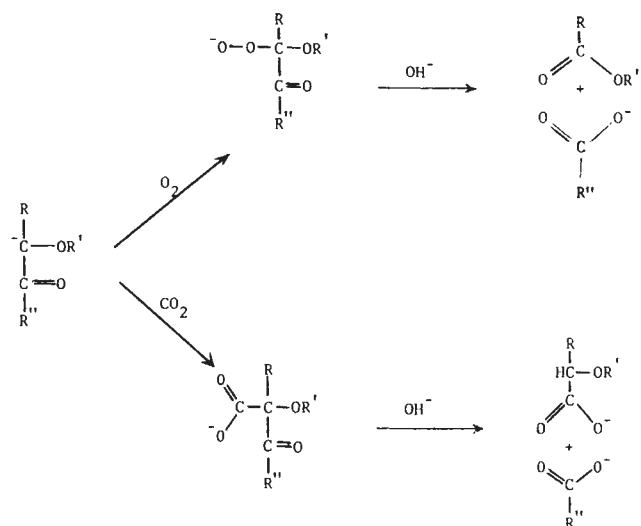
Plant Photorespiration—An Inevitable Consequence of the Existence of Atmospheric Oxygen

THE primary event in the glycolate pathway¹, the metabolic sequence responsible for the phenomenon of photorespiration in plants², is the oxygenation of ribulose 1,5-diphosphate (RuDP) to phosphoglycolate and 3-phosphoglycerate³⁻⁵. This reaction appears to be catalysed by the enzyme RuDP carboxylase⁴. When ¹⁸O₂ is supplied, incorporation of one atom of ¹⁸O into the carboxyl group of one of the products only, namely phosphoglycolate, is observed. The other atom of the oxygen molecule is exchanged with the medium⁵. Similarly, intact spinach leaves incorporate isotopic oxygen, supplied as O₂, into the carboxyl groups of the glycolate-pathway intermediates, glycine and serine⁶. The purpose served by photorespiration has remained an enduring riddle⁷. Superficially, it would seem that oxygenation of RuDP must be deleterious to the photosynthetic process, necessitating the glycolate pathway to recoup as much of the phosphoglycolate carbon as possible with one quarter of it being lost as CO₂ at the glycine→serine conversion. Observations that net photosynthesis and plant growth in general are stimulated in oxygen-depleted atmospheres concomitantly with a suppression of photorespiration^{8,9} reinforce this concept.

We suggest that oxygenation of RuDP, and therefore photorespiration, is chemically unavoidable when photosynthesis occurs in an oxygen-containing atmosphere because an intermediate generated during the reaction of RuDP carboxylase with RuDP is susceptible to attack by oxygen. We suggest that this intermediate involves the enolate anion of RuDP, the carbanion formed at C(2) being susceptible to attack by either CO₂ or oxygen. There is chemical precedent for both carboxylation and oxygenation of carbanions. For example, 2-methoxy-2-phenyl acetophenone reacts with both oxygen¹⁰ and CO₂ (refs. 11, 12), presumably by means of the enolate anion, the structure of which resembles that of RuDP. The carboxylation of 2-methoxy-2-phenyl acetophenone has been suggested as a model reaction for RuDP carboxylase¹².

This hypothesis predicts that all RuDP carboxylases using the proposed mechanism, regardless of source, should also have oxygenase activity. If RuDP cannot be carboxylated other than as the enolate anion, then concomitant oxygenation is unavoidable if oxygen is present. If this is the case, attempts to reduce or eliminate photorespiration in crop plants by genetic manipulation are unlikely to succeed.

Because RuDP carboxylase has such an essential role in photosynthetic CO₂ fixation, this activity would have been conserved during evolution regardless of its inefficiency. Because the challenge presented by rising atmospheric oxygen concentration could not be met by modification of the carboxylase to prevent oxygenation, adequate means for the



In 2-methoxy-2-phenyl acetophenone $\text{R}, \text{R}'' = \text{C}_6\text{H}_5$, $\text{R}' = \text{CH}_3$.

In ribulose, 1,5-diphosphate
 $\text{R} = \text{CH}_2\text{OPO}_3^{2-}$, $\text{R}' = \text{H}$, $\text{R}'' = \text{CH}(\text{OH})\text{CH}_2\text{OPO}_3^{2-}$

disposal of the phosphoglycolate waste product would have been required. In aquatic organisms dephosphorylation and excretion of glycolate may have been employed initially and many modern algal species still do this under certain conditions. A different mechanism would have been required, however, by terrestrial plants and we suggest that the glycolate pathway has evolved to fulfil this requirement.

Although plants which use the C₄ dicarboxylic acid (C₄) pathway do not exhibit the external manifestations of photorespiration^{9,13}, oxygen exchange studies indicate that photorespiration does occur¹⁴ and it must be concluded that the CO₂ released is refixed before it can escape from the leaf. Thus C₄ plants do not constitute an exception to the present hypothesis. The rate of photorespiration in some C₄ pathway plants, however, as judged by the activities of glycolate-pathway enzymes, is probably less than that in plants with the Calvin cycle^{15,16}. It has been suggested that this is because C₄ plants concentrate CO₂ at the site of RuDP carboxylase, thus allowing carboxylation to compete more effectively with oxygenation for the available RuDP³. The demonstration of an intermediate internal pool of CO₂ in C₄ plants¹⁷ supports this idea.

A similar situation may occur in plants with crassulacean acid metabolism (CAM) where deacidification in the light is accompanied by release of CO₂ from malic acid¹⁸. As the stomata are closed at this stage of the diurnal cycle, the CO₂ concentration in the leaf may rise considerably above ambient, thus achieving the same stimulation of carboxylation at the expense of oxygenation that is suggested for C₄ plants.

The metabolic modifications of both the C₄ pathway and CAM might therefore be viewed, at least in part, as adaptations aimed at minimizing the inevitable deleterious effects of atmospheric oxygen on the photosynthetic process.

G. H. LORIMER

Forschungsstelle Vennesland der Max-Planck-Gesellschaft,
 1 Berlin 33 (Dahlem), Harnackstrasse 23, Germany

T. J. ANDREWS

Department of Environmental Biology,
 Research School of Biological Sciences,
 Australian National University, PO Box 475, Canberra City,
 ACT 2601, Australia

Received January 16, 1973.

¹ Tolbert, N. E., in *Photosynthesis and Photorespiration* (edit. by Hatch, M. D., Osmond, C. B., and Slatyer, R. O.), 458 (Wiley, New York, 1971).

² Jackson, W. A., and Volk, R. J., *Ann. Rev. Plant Physiol.*, **21**, 385 (1970).

- ³ Bowes, G., Ogren, W. L., and Hageman, R. H., *Biochem. Biophys. Res. Commun.*, **45**, 716 (1971).
- ⁴ Andrews, T. J., Lorimer, G. H., and Tolbert, N. E., *Biochemistry*, **12**, 11 (1973).
- ⁵ Lorimer, G. H., Andrews, T. J., and Tolbert, N. E., *Biochemistry*, **12**, 18 (1973).
- ⁶ Andrews, T. J., Lorimer, G. H., and Tolbert, N. E., *Biochemistry*, **10**, 4777 (1971).
- ⁷ Goldsworthy, A., *Nature*, **224**, 501 (1969).
- ⁸ Turner, J. S., and Brittain, E. G., *Biol. Rev.*, **37**, 130 (1962).
- ⁹ Bjorkman, O., in *Photosynthesis and Photorespiration* (edit. by Hatch, M. D., Osmond, C. B., and Slatyer, R. O.), 18 (Wiley, New York, 1971).
- ¹⁰ James, T. H., and Weissberger, A., *J. Amer. Chem. Soc.*, **59**, 2040 (1937).
- ¹¹ Proske, B., thesis, Univ. of Heidelberg (1956).
- ¹² Cramer, F., and Proske, B., *Angew. Chem.*, **68**, 120 (1956).
- ¹³ Hatch, M. D., and Slack, C. R., in *Progress in Phytochemistry* (edit. by Reinhold, L., and Liwischitz, Y.), **2**, 35 (Interscience, London, 1970).
- ¹⁴ Volk, R. J., and Jackson, W. A., *Plant Physiol.*, **49**, 218 (1972).
- ¹⁵ Osmond, C. B., and Harris, B., *Biochim. Biophys. Acta*, **234**, 270 (1971).
- ¹⁶ Huang, A. H. C., and Beevers, H., *Plant Physiol.*, **50**, 242 (1972).
- ¹⁷ Hatch, M. D., *Biochem. J.*, **125**, 425 (1971).
- ¹⁸ Ranson, S. C., and Thomas, M., *Ann. Rev. Plant Physiol.*, **11**, 81 (1960).

Interspecific Hybridization in *Cardium*

BOYDEN¹ has shown that cross-fertilization takes place between the cockles *Cardium edule*, Linné, and *Cardium glaucum*, Bruguière. He used, as the criteria for successful hybridization, either the production of rotating trochophores, still within the confines of the egg sheath, or free-swimming veligers; no attempts were made to rear the larvae.

Table 1 Percentage Success of Cross-fertilizations between Individuals Originating from: a, Geographically Distinct Populations; b, a Mixed Cockle Population

a													
Cross	1	2	3	4	5	6	Experiment 7	8	9	10	11	14	Further development
Intraspecific	75	35	90	2	72	24	50	10	85	40	70	96	Majority to metamorphosis
Interspecific	8	5	10	0	<1	7	<1	0	20	0	15	0	All died
Control	0	0	0	0	0	0	0	0	0	0	0	0	—
b													
Cross	9	10	11	12	13	15	Experiment 16						
Intraspecific	85	40	70	51	17	71	18						
Interspecific	12	0	0	0	0	30	4						
Control	0	0	0	0	0	0	0						
													Further development
													Majority to metamorphosis
													Those of experiment 15 metamorphosed
													—

Female parent of interspecific crosses: experiments 1, 2, 5 and 6, *C. edule*; remaining experiments, *C. glaucum*.

Origin of individuals used in the experiments: *C. edule*, Shoeburyness, Essex; Ellanore, Chichester Harbour, W. Sussex; River Crouch, Essex; *C. glaucum*, Widewater, W. Sussex; River Crouch, Essex.

Galtsoff and Smith² were able to cross-fertilize two oyster species, *Crassostrea virginica* and *Crassostrea gigas*, and obtained what appeared to be normal, healthy veliger larvae. Davis³ also successfully cross-fertilized these two species of *Crassostrea*, but although the larvae appeared normal and vigorous in behaviour, none survived beyond the tenth day of development.

A recently developed method of reliably rearing the larvae of *C. edule* and *C. glaucum* through to metamorphosis has enabled fully controlled cross-fertilizations to be conducted in the laboratory. This allows not only the determination of the success rate of cross-fertilization, but also the ability of the resultant larvae to survive.

Unfertilized ova and spermatozoa were obtained by inducing isolated, sexually mature cockles of both species to spawn in the laboratory. The experimental cross-fertilizations were carried out in 250 ml tall-form beakers, in which 5,000 to 8,000 ova were inseminated, using 1.5 ml of spermatozoa suspension prepared by diluting the entire produce of a single male in 500 ml of sterile seawater. The ova used in all the experiments were the results of normal spawning and in no cases were artificially stripped ova used.

Insemination was effected as soon after discharge as possible, thereby minimizing post-spawning deterioration effects.

Each experiment consisted of duplicate cultures—one pair with the intraspecific cross, one pair with the interspecific cross and one pair of control cultures in which no spermatozoa were added to the ova. Maintenance of control cultures of uninseminated ova was essential for two reasons. First, although the seawater used in the test cultures had been carefully membrane filtered, it was impractical to keep the adult stocks in water thus treated, so there was always the possibility of fertilization by unwanted spermatozoa already present in the water. Second, the occurrence of hermaphroditic individuals is not unknown so the possibility of self-fertilization could not be overlooked.

After incubation for 24–48 h, during which time the fertilized ova had undergone sufficient development to be easily recognized, 100–200 eggs were removed and examined to determine the numbers of fertilized and unfertilized eggs. Further development was then allowed to take place, the cultures being maintained in optimum conditions for growth.

The results of these cross-fertilization experiments are shown in Table 1. It becomes immediately apparent from these figures that cross-fertilization is possible between *C. edule* and *C. glaucum*, further substantiating the findings of Boyden¹. Examination of the results, however, reveals that the success rate of the interspecific crosses is small when compared with the intraspecific fertilizations. Subsequent rearing of the hybrid larvae met with little success in most of the attempted crosses; in a single case, however (experiment 15), the larvae lived long enough to metamorphose into adults. In general, cross-fertilizations between species originating from a mixed population (for example, the River

Crouch) tended to be less successful than crosses between species from different geographic locations. The poor survival of hybrid larvae is of particular significance, especially in mixed cockle populations where it could result in the reduction of the reproductive capability of the two component species.

In mixed populations, individuals bearing apparent intermediate morphological characteristics do occur. Although limited, the successful rearing of hybrid larvae suggests that these intermediates are in fact true hybrids, and that their morphology is determined genetically and not simply as the result of environmental influence.

I thank Dr David Urry for help. This work was conducted during the tenure of a research studentship at the Hatfield Polytechnic.

P. KINGSTON

Dove Marine Laboratory,
Cullercoats, Northumberland

Received January 24; revised March 21, 1973.

¹ Boyden, C. R., *J. Mar. Biol. Ass. UK*, **51**, 605 (1971).

² Galtsoff, P. S., and Smith, R. O., *Science*, **76**, 371 (1932).

³ Davis, H. C., *Science*, **111**, 522 (1950).

BOOK REVIEWS

Bayesian Psychology

In Defence of Empirical Psychology. By Donald E. Broadbent. Pp. ix+221. (Methuen: London, February 1973.) £2.90.

ON the wall of a colleague's laboratory there is a sign which reads "My mind is made up. Please don't confuse me with facts". In this book, which contains the six William James Lectures given by Donald Broadbent at Harvard University in 1971 along with four other lectures given from 1965 to 1970, the subtext seems to be—my mind is made up, don't confuse me with rational or theoretical argument. The common theme of the lectures is that facts must reign supreme in science and in society. The empirical method is the surest way to truth and understanding, and the empiricist's epistemology and his *weltanschauung* are in the highest tradition of Anglo-Saxon history and culture.

Broadbent argues that a person's scientific, political, moral and social lives are all of a piece. The way one goes about one's intellectual business is consistent with the way one goes about one's various other pursuits. Empiricists of Broadbent's stripe are different (and by implication better?) sorts of people than are rationalists, not only as scientists, but as social and political animals as well. One wonders what sorts of people theoretical mathematicians are.

The true empiricist seems to be one who subscribes wholeheartedly to a Bayesian view of life. As a scientist, the best strategy is to consider, at all times, the relative weight of evidence for alternative theories rather than to seek evidence in support of one favoured theory. As humans, Bayes's theorem seems to characterize much of human involuntary behaviour, particularly decision-making under conditions of uncertainty, and Broadbent argues that "... our voluntary behaviour ought to conform to ..." the theorem as well (page 11). The theorem itself, proposed by the Reverend Thomas Bayes in 1736, simply provides a mathematical procedure for calculating the likelihood that one of several alternative hypotheses is correct. Broadbent's Bayesian outlook on life is that one should always

consider the relative weight of evidence for at least two alternatives, be they courses of action, policy decisions or scientific hypotheses. Put crudely, one should try to calculate the odds that any particular alternative is correct relative to other alternatives, and then bet the odds. Query—is this strategy peculiar to empiricists, and do all empiricists adopt this strategy either as a way of doing science or of living in the world?

Broadbent implies that this is so, and characterizes his own work, and his life as well, as exemplifying this philosophy. Occasionally, as in his critique of some theoretical linguists, he endows proponents of opposing views with certain qualities inferred from his personal model of human personality... if someone is not an empiricist in his work, then he tends to be polemical and emotional about his work, and in other aspects of his life as well. For example, anti-empiricists (and only anti-empiricists) argue that it is important for one's work to be relevant to broad social issues (rather than to the design of, say, aircraft instruments), and their attitudes are seen as an imminent danger to society as a whole. All in all, this line of argument strikes me as unnecessarily *ad hominem*. The substantive issues in cognitive psychology and Broadbent's views on those issues can be treated far more convincingly without appeals to the good, the true and the beautiful.

Fortunately, Broadbent's own work does not conform to the extreme empiricist ideals he would have us embrace. He refers to his taking up a major portion of his Myers lecture by conceptual analysis rather than by experimental results as a "horrible example" (page 207). Nevertheless, his principal contribution to psychology has been conceptual. His most influential book, *Perception and Communication*, is only trivially empirical. Its impact, which in no small way helped to bring about the cognitive revolution in psychology, derived primarily from his compelling conceptual analysis of the brain as a processor of information, not from a set of compelling "facts" and data. Indeed, the predominant reaction of many stimulus-response behaviourists at the time was that the book was hopelessly mentalistic.

In short, the book reveals much of Broadbent the person as well as Broadbent the scientist, and the two are not as congruent as he seems to believe. But then, none of us is as self-consistent or rational as we tend to feel we are. To those of us involved in experimental psychology this book provides an interesting and sometimes revealing picture of one of our more distinguished colleagues. To those contemplating a career in science, I would add this note of caution: do as Broadbent does, not as he says he does. SAM GLUCKSBERG

Air-Sea Interface

Atmosphere-Ocean Interaction. By E. B. Kraus. Pp. viii+275. (Clarendon: Oxford; Oxford University: London, April 1972.) £7.50.

THIS book has the avowed purpose of telling meteorologists something about relevant studies in oceanography and *vice versa* and also of interesting physicists in the physics of the environment. There can be no doubt that both these tasks need undertaking and the author's contribution to this end is to be welcomed. The book opens with a condensed review of the fluid mechanics needed for treating the dynamics of large-scale flows. This is a useful recapitulation of the subject and serves as a source of reference to the relevant equations for the rest of the book. Following this, the physical scene is set with an account of the properties of sea water on one side of the air-sea interface and of moist air on the other, with the transfer processes between them. A chapter on radiation gives a clear and up-to-date summary of the vicissitudes of solar radiation, in its passage through the atmosphere and into the surface layer of the sea, and also of terrestrial radiation.

The subject of surface waves has a long history of investigation and a voluminous literature but it is one in which important advances are still being made. The chapter on this subject gives an account of recent theories of wave generation by wind and an interesting feature is the attention paid to the properties of the flow in the air over waves as well as in the water. The next two chapters deal with turbulent transfer near the interface, in which the coupled

transports of heat and moisture in relation to that of momentum and energy are of particular concern, and processes in the planetary boundary layers where Coriolis effects become important. A parallel treatment of effects in both the atmospheric and oceanic layers is followed as far as possible.

Three-dimensional interactions form the subject of the last, and longest, chapter and in a sense the whole book has been preparing the ground for this wide field of study. The interrelation of sea-surface temperature and the dynamics of the tropical atmosphere, the development of hurricanes and the inter-tropical convergence zone are among the topics treated. The properties of inertio-gravity and planetary waves in the ocean are discussed in relation to the response of the ocean to storms and to seasonal changes in the wind regime. It will be seen that the appearance of this book is particularly opportune at a time when GARP (the Global Atmospheric Research Programme) is getting into its stride and GATE (GARP Atlantic Tropical Experiment) is in active preparation.

K. F. BOWDEN

Coastal Habitats

Ecology of Salt Marshes and Sand Dunes. By D. S. Ranwell. Pp. xiv + 258 + 16 plates. (Chapman and Hall: London, December 1972.) £4.60.

SINCE the emergence of ecology as a distinct discipline, both sand dunes and salt marshes have exercised an almost magnetic attraction for plant ecologists. It is not difficult to recognize the cause of this attraction. Both types of habitat have very distinctive types of vegetation which contain a high proportion of species restricted to them, and many of these species show obvious adaptation to the special conditions which prevail. On salt marshes, particular attention has been given to the causes of zonation and to the adaptation of plants to salinity and to periodic immersion in seawater, while on sand dunes interest has generally been focused on adaptation to movement of sand and on mineral nutrition. It has been generally accepted that both habitats provide clear evidence of vegetational succession and indeed sand dunes were among the habitats where this process was first recognized and described in the early years of this century.

It has long been recognized that a full understanding of both habitats requires knowledge of coastal physiographic processes and it now seems likely that the establishment of flowering plants on freshly deposited marine sediment or wind-blown sand is dependent on the

activities of the soil fauna and of micro-organisms.

In recent years, both habitats have acquired a less welcome reason for attracting the attention of ecologists. This is the vulnerability of salt marshes to pollution of estuarine and coastal waters and of sand dunes to destructive erosion apparently activated simply by the wearing away of their vegetation by holiday-makers.

The success of interdisciplinary investigations of both habitats, and the recognition of their vulnerability to damage, fully justify a new appraisal of the state of our knowledge of their overall ecology. Dr Ranwell has set out to do this. Certainly a lot of information is presented but often in the style of a summary without any real synthesis. For example, the description of physiographic types of dune-systems is written almost in the form of notes. Maps and diagrams would allow a reader, unfamiliar with more than a few sites, to grasp the full range of diversity and its probable causes. One of the four sections of the book is devoted to the effects of man and draws attention to many aspects of the ecology of dunes and salt marshes which were either unrecognized, or regarded as less important, in accounts written at a time when damage was less obvious and conservation was not uppermost in our minds.

Although the title of the book includes the unqualified word ecology, the treatment of both animals and micro-organisms is brief and superficial. Rabbits, once so important, are discussed in a few short paragraphs and there is almost no mention of birds. The few pages devoted to insects are largely concerned with energy-flow, not with their biology, and other groups of invertebrates are scarcely mentioned. There are scattered references to micro-organisms but such interesting groups as the bacteria which are concerned with oxidation-reduction processes in waterlogged soils of salt marshes are not discussed.

The book is primarily concerned with plant ecology but, even in this, the author deliberately restricts himself in a way which seems to lead to a confused presentation. The overall successional relationships are omitted on the grounds that they are often an oversimplification. But this is true of most ecological generalizations and not least of attempts to formulate flow diagrams of energy or substances through ecosystems. These are generally based on very sparse measurements of fluxes and inadequate knowledge of pathways. Then all descriptions of the composition of vegetation are excluded on the doubtful assertion that "the presence or absence of most species in a habitat is irrelevant to the great majority of other species (includ-

ing man) in the habitat". One must wonder what does control the composition of vegetation and the population dynamics of animals. As a consequence of these two omissions much of the discussion and analysis has no clear focus. It is not easy to reconcile this rejection of accurate description with the plea on page 181 to give more attention to detailed studies of parts of ecosystems in order to understand the whole.

Much of the book seems symptomatic of a deep seated confusion in the minds of many ecologists. Description may not bring understanding but at least it defines what has to be understood. Analysis and many numerical data derived from analysis are often still purely descriptive. What is a model but a form of description? The equation on page 84 which is claimed to be a model of accretion and has its constants given to three significant figures, but fails to give the units of measurement, shows convincingly that numbers alone do not improve bad science.

C. D. PIGOTT

Food Technology

Health and Food. Edited by G. G. Birch, L. F. Green and L. G. Plaskett. Pp. xi + 224. (Applied Science: Barking, Essex, 1972.) £4.20.

THIS is a collection of papers read at a meeting organized by the authors as one of a series of annual international symposia of the National College of Food Technology, University of Reading.

It was intended as a discussion of the potential health problems that may arise from changes taking place in our food because of the rapid development of food technology. Almost all our food—a figure of 98% of the total energy intake has been suggested—passes through the hands of the food processor. Two questions were to be asked: are manufactured foods safe from the point of view of additives, processing changes, pesticide residues and so on; and what should be regarded as a good diet?

As might be expected, no conclusions were reached but the topics covered included discussions of consumer attitudes to processing, hazards of natural foods, methods of testing additives, legal aspects, pesticide residues in baby foods, yeast protein, ready-made meals, clinically designed foods, space foods, diet and longevity, problems arising with new foods. Speakers were drawn from industry and the academic field and from the United States, Holland, Poland and France as well as the United Kingdom.

As the titles indicate, the symposium was somewhat diffuse and unstructured

but the material including the discussions provides useful material for continuing discussion and argument.

A. E. BENDER

Phase Transitions

Phase Transitions and Critical Phenomena. Edited by C. Domb and M. S. Green. Volume 1. *Exact Results*. Pp. xv + 506. (Academic: London and New York, December 1972.) £10; \$31.

THIS is the first in a projected series of at least four volumes on the theory of phase transitions, intended to serve as a standard reference text for some time to come, particularly for graduate students. This intention sounds a little like trying to catch a rainbow, so rapidly is knowledge advancing in this area, but the book can stand on its merit as a set of high-quality review articles.

The book begins with two prefaces (one to the series, one to this volume) by the editors, and an introductory note by C. N. Yang. In only a few pages, they give a good overall view of the present state of the subject, how it got there, and how it might conceivably develop in the future.

The first of the review articles, by R. B. Griffiths, is about rigorous results and theorems. As we have come to expect from this author, it is a model of clarity. It also reveals his sure touch in dealing with the perennial dilemma facing all authors of such articles: whether to risk wearing out the reader by including too much mathematical detail, or risk losing conviction and coherence by not including enough. Connoisseurs will also enjoy this article for some entertaining misprints, such as "by purring bounds on the magnitude of the interaction energy" on page 24, and a real collector's item (too complicated to quote here) in equation (2.C2) on page 19.

There follow four shorter articles. First, J. Ginibre supplies some of the mathematical detail omitted from Griffiths's article, giving a clearly written and fairly complete account of the proof that infinite-volume correlation functions exist and are analytic for sufficiently dilute quantum systems. Next, G. G. Emch describes the C*-algebraic approach to phase transitions. This approach uses a great deal of special mathematical formalism, and it is often difficult for the uninitiated to understand either the meaning of the definitions and theorems or what purpose they serve. The great virtue of this article over most articles in this field is the effort that has gone into explaining these very points.

The next article, by C. J. Thompson, treats one-dimensional models with short-range interactions. Such systems never show phase transitions, but they do fit the secondary theme of this

volume, exact results. The fourth article of this group, by H. N. V. Temperley, surveys the methods that have been devised for calculating exactly the thermodynamic properties of the two-dimensional Ising model (two-state "atoms" on each vertex of an infinite square lattice). Their variety and interest are a testimony to the vitality of this problem, first solved by Onsager (1944) in a *tour de force* of mathematical technique.

A slightly longer article, by I. Syozi, is a *tour de force* of a different kind; it shows how Ising models on an astonishing variety of two-dimensional lattices can be solved by transformations and extensions of Onsager's result. The most picturesquely named lattice is the "Asanoha, or hemp-leaf", lattice; the physically most surprising result is that some of the more complicated lattices treated can have three or even five transition temperatures.

The last article, by E. Lieb and F. Y. Wu, with insertions by E. Barouch, D. B. Abraham and R. J. Baxter, is almost a book in itself. It deals with the many variants and generalizations of the "two-dimensional square ice" model first solved by Lieb in 1967. Unlike the others in the book, this is an article for the specialist, going into great mathematical detail and presenting many results that seem to be new.

The volume can be strongly recommended to anyone with £10 to spare and an interest in recent research in the theory of phase transitions.

O. PENROSE

Semiconductors

Theory of Electrical Transport Semiconductors. By B. R. Nag. Pp. vii + 227. (Pergamon: Oxford and New York, December 1972.) £4.50.

THE theory of electron transport in semiconductors has made great strides forward over the last six or seven years. Monte Carlo and iterative computational methods have been developed and successfully applied to a large number of semiconductor problems. Most researchers feel that the way forward lies in the exploitation of these methods. It is to be regretted that Professor Nag is not of that company. The new methods are dismissed in a few lines on the last page of the text with the implication that they are inferior to approximate analytic methods and do not help to build up physical insight. It would be more correct to say that the new computation procedures provide exact solutions to the Boltzmann equation and leave the researcher free to concentrate on the physical processes involved. Moreover, it is hard to imagine a calculation more replete with shafts of physical insight than a Monte Carlo

simulation which follows individual electron trajectories.

The book under review is concerned with electrical conductivity theory as it existed before the introduction of modern computational methods. Electron states are discussed in an elementary way in chapter 1 and phonons and electron-phonon interactions are similarly treated at the beginning of chapter 2. The last twenty pages of chapter 2 provide a useful summary of the significant scattering processes in semiconductors. The bulk of chapter 3 is concerned with the relaxation time approximation and the relaxation times for the various scattering mechanisms are calculated in chapter 4. Experimental techniques are briefly reviewed in chapters 5 and 6 and chapter 7 is devoted to the application of semi-analytic techniques to high-field transport problems.

The balance of the book is poor. Considerable space is devoted to elementary notions concerning electrons and phonons which are adequately treated in a number of well known texts. Basic transport theory, on the other hand, is skimmed over very lightly. No discussion is given of either thermal transport phenomena or the Onsager relations. Boltzmann's equation is never stated properly; both the time derivative of the distribution function and Liouville's theorem are omitted from the derivation of the equation and there is no discussion of the limits of its validity. Variational methods for solving the linearized Boltzmann equation are given just two pages of text with no derivation or discussion of the functional involved. There are also a number of worrying errors in the text. The first examples appear on page two with the statements that the momentum of an electron is given by $-\hbar\nabla\psi$ and its energy is $\hbar\partial\psi/\partial t$.

The book is aimed at graduate students and new entrants to the field of semiconductor transport theory. There is certainly scope for a new book for this audience which carefully presents the basic theory, deals with the recent advances and points the way forward. The text under review does none of these things and is altogether too slipshod to be recommended.

P. N. BUTCHER

Antibiotic Action

The Molecular Basis of Antibiotic Action. By E. F. Gale, E. Cundliffe, P. E. Reynolds, M. H. Richmond and M. J. Waring. Pp. xviii + 456. (Wiley: London and New York, 1972.) £8.

THIS book deals mainly with studies on the biochemical and molecular basis of antibiotic action. As indicated by the

authors in the preface, however, the book is also concerned with other drugs and compounds whose cellular targets are similar or somehow related to those of the antibiotics.

The volume is divided into eight chapters beginning with a brief outline of the Ehrlich concept of chemotherapy including a short account of the development and mode of action of arsenical drugs, sulphonamides and early work on antimetabolites. There is a brief description of the biochemical targets for drug action, selectivity and resistance, antibiotic inhibitors of peptidoglycan synthesis, drugs and compounds altering the function of the cytoplasmic membrane, inhibitors of nucleic acid synthesis, inhibitors of the ribosome function and bacterial resistance to antibiotics. The book ends with a discussion on some possible experimental approaches to the antibiotic mode of action (double blockage, modification of existing antibiotic nuclei, and the use of alkylating agents).

The emphasis is on basic research, but the authors usually dedicate most of their contributions to drugs and antibiotics important for clinical use as antibacterials. Perhaps this is the reason for the omission of antibiotics acting on respiration and oxidative phosphorylation such as antimycin A, piericidin A, usnic acid, oligomycin, rutimycin and aurovertin.

It is stated in the preface that each author has written individually the chapter in which he is specialized and this has led to some lack of uniformity. For example, when dealing with inhibitors of nucleic acid synthesis, agents interfering with nucleotide metabolism are briefly described besides those drugs inhibiting either the enzymic process of nucleic acid synthesis or the template function of DNA. On the other hand, inhibitors of aminoacyl-tRNA formation such as borrelidin and furanomycin, and inhibitors of formyl-methionyl-tRNA_f formation such as aminopterin and trimethoprim, are not mentioned in chapter 6. In most cases, however, uniformity of style has been successfully achieved. All the contributors are, or have been for many years, members of the same sub-department of chemical microbiology in Cambridge working under the same head and it is very obvious the influence of this stage of their career in their style.

The book is well written and very carefully edited. The lists of references in each chapter are well selected and the field is well covered until August 1971; even many data not yet published at that time are included. The book is very pleasant to read and should be very useful not only to specialists on the increasing fields of antibiotic mode of action, but also to workers involved in

teaching or those wishing to use correctly antibiotics as tools in research.

D. VÁZQUEZ

Oceanography

Seventy-Years Agrowing: a History of the International Council of Exploration of the Sea. By A. E. J. Went. Pp. 252. (International Council for the Exploration of the Sea: Charlottenlund Slot, Denmark.)

THE International Council for the Exploration of the Sea was set up by eight European countries. Eight more, and Canada, joined later. There had already been national and international agreement to ensure good order among fishermen, but the great expansion of the industry following the introduction of steam propulsion, development of the otter trawl, and extension of markets by rail transport had brought fears of overfishing. Initiative for systematic study, coming from Scandinavia, was supported in the UK by prominent scientists, by the National Sea Fisheries Protection Association and by the Sixth International Geographical Congress meeting in London. Otto Pettersson, one of the most dedicated marine researchers of the day, helped by Nansen, persuaded the Swedish Government to call a planning conference in 1899 and the Council met for the first time in 1902. It has met annually for 70 years with some interruption during the two world wars.

From 1902 to 1908 it had its own Central Laboratory which proved a veritable cradle for oceanography, setting the pattern for half a century. The outlook then narrowed a little. While exploration added continually to our knowledge of the plankton and fishes of the Atlantic Ocean, the North Sea and the Baltic Sea, immediate problems such as mesh regulation, decline of herring stocks, dependence of recruitment on parent stock and building up reliable statistics were the main purpose. Progress was necessarily slow, but the Council has often been able to give advice of permanent value to the industry and has been the parent of a number of valuable conventions and commissions.

Dr Went deals with the scientific work and its achievements, but mostly in the context of organization, committees and personalities. He describes the early meetings as "rather family affairs with a few outsiders" and one sometimes wonders if the circle has been too restricted. It could, for example, have taken a more active part in the rapid widening of interest in marine science after the Second World War. It has since organized IGY work in "the ICES area", and arranged a symposium on physical variability, bringing an

unusually wide range of scientists to one of its meetings.

It is clear that the Council has had excellent service from the small administrative and technical staff working at its headquarters in Copenhagen.

G. E. R. DEACON

Regulation in Cells

Control Mechanisms and Protein Synthesis. By S. D. Wainwright. Pp. vii + 550. (Columbia University: New York and London, November 1972.) £9.40.

THE regulation of protein synthesis has long been one of the exalted topics in biology. Twelve years after its publication, the operon hypothesis has become firmly established as the primary mechanism for the negative control of gene expression in many bacterial systems. In spite of the obvious feasibility of the operon model as a general regulatory concept, it remains obscure to what extent this mechanism is employed in the more complex organisms. Incisive information has proved difficult to obtain because of the dearth of eukaryotic systems suitable for both genetic and biochemical analysis of the necessary detail. It is apparent, however, that eukaryotes have various control mechanisms which have no counterpart in prokaryotic systems.

Dr Wainwright's aim in *Control Mechanisms and Protein Synthesis* is to describe and assess the current state of our knowledge in this complex field. His view of the subject is broad, ranging from RNA-containing bacteriophages with only three genes to hormonal effects in metazoans. The treatment is detailed, though a more chemical approach might have been appreciated in those few cases where the molecular interactions are well enough understood. In places I found the sequence of chapters surprising. Thus the introductory chapter, "The System Regulated", a detailed and comprehensive account of the mechanism of translation, is followed by a contrastingly superficial chapter on differentiation and gene amplification. The synthesis of messenger RNA, often itself "the system regulated", is saved until chapters 5 and 6. Later chapters deal with molecular differentiation and antibody formation, devoting considerable space to models and hypotheses. A final chapter on recent developments ensures that the coverage is very up to date.

Dr Wainwright presents a balanced, if unexciting, account of the regulation of protein synthesis from the biochemist's viewpoint. The real strength of his book is its extraordinarily comprehensive bibliography which, together with the excellent index, will make it an invaluable guide to an increasingly daunting literature. W. J. BRAMMAR

INSTRUMENT REVIEW

Analysis for Production Control

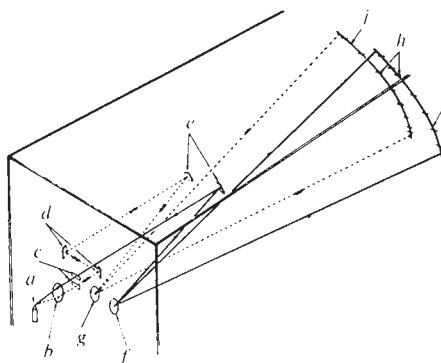
Polyvac E1000 System (Rank Precision Industries Ltd, from about £25,000).

PRODUCTION control of iron and steel, and of other metals and alloys, requires rapid and precise analysis of the molten base materials. Particular care must be taken that the composition of the sample does not change during the solidifying process, and that the time between sampling and analysis is as short as possible. Classically, analyses were made by wet chemistry, that is, the sample was dissolved, the element of interest separated and its concentration determined. Since the Second World War, physical methods of analysis, which are more rapid and do not involve highly trained staff, have increasingly displaced wet chemistry from the industrial routine analytical laboratory. The most common are spectroscopic techniques, in which the atoms of the sample are electrically excited to emit radiation, which is separated into its components by prisms, gratings or by other means, depending on the kind of radiation emitted (visible, ultraviolet or X-ray). The intensity of emission of a particular wavelength can then be converted into concentrations per cent by weight of the emitting element.

In the field of emission spectroscopy, a new vacuum direct reading instrument has recently been developed. This analysis system, consisting of the excitation unit, the spectrometer, a computer and a complete software package, makes it possible to carry out complete analyses of twenty-five elements within one minute. The first Polyvac E1000 system was installed at the Stainless Steel Works of Gebr. Böhler and Co. AG at Düsseldorf, Germany, and has been in continuous operation for the past year.

The excitation unit FS1630 used in the Polyvac E1000 system makes short excitation times possible by its feature of a variable discharge frequency. Repetition rates of 50, 100, 200, and 400 s^{-1} can be selected, resulting in a shortening of the sparking time from approximately 25 s at 50 Hz to about 3 s at 400 Hz, with the same efficiency of light production. Another important improvement in the FS1630 excitation unit is that individual discharges can be counted, so that the sparking is terminated when a preselected number of discharges have occurred. Independent of the internal standard (which is usually a matrix similar to the sample examined), the simultaneous analysis of all

given elements of a sample has thus become possible at constant analysis times. In the past, analyses were made either against the internal standard, using variable analysis times, or against different standards using constant analysis times; normalizing to 100 per cent was indirect and involved calculations.



The optical path of the Polyvac E1000. *a*, Light source; *b*, condenser lens; *c*, entrance slits; *d*, periscope mirrors; *e*, plane mirrors; *f*, grating A; *g*, grating B; *h*, exit slits; *i*, focal curve A; *j*, focal curve B.

The second step towards reducing analysis time was made by integrating a digital computer into the system because, in the case of complex alloys, extensive calculations are required. The signals from the secondary electron multipliers are converted into concentration values (per cent by weight). Because a complex material omits a large number of spectral lines, some interference from neighbouring lines occurs, in spite of exact positioning of the exit slits. Clearly, this "noise level" depends on the composition of the sample and may vary considerably. It can, however, be corrected for, although a detailed discussion of inter-element and matrix effects would be beyond the scope of this review. To obtain an accurate analysis, all interfering factors must be recognized, calculated and corrected for. Only then can the first normalization to 100 per cent be made, a process which the computer can carry out within a fraction of a second of sparking.

The Digital Equipment computer PDP8/e, which is an integral part of the Polyvac E1000 system, not only performs these calculations but also controls the analytical procedure and monitors the maintenance of the given

instrumental parameters. It requires a well developed software package; that used in the Polyvac E1000 system has performed very well since its installation. Some uncertainty was felt originally about replacing the conventional electronic controls by a computer. In retrospect, after a year of round-the-clock operation of the system, it can be said that the decision taken was the right one—the computer did not fail once during that entire period. Control and monitoring functions cover the control of electrical parameters, the argon flow, pre-spark and spark periods, and recalibration. The recalibration process is also fully automatic; it guarantees the analytical reproducibility of the method and is an important part of the system.

An essential component for accurate analyses is the spectrometer. The Figure shows schematically the optical path of this advanced apparatus, which offers the advantages of twin entrance slits, diffraction gratings, and exit slit plates. Thus the most intense spectral line for any element can be selected. For very complex materials with complex spectra it is obviously advantageous to have a combination of two suitable gratings, making it possible to analyse simultaneously spectral lines within the wavelength range from 1,596 Å to 8,640 Å or, depending on the problem on hand, to utilize the maximum reciprocal dispersion over the whole range.

The exit slits are engraved in the slit plate; by aligning the plate for one line, all other lines are aligned automatically, thereby saving substantial setting-up time. When the analytical programme is changed, it is fairly easy to replace one slit plate by another with a different exit slit arrangement. Each slit plate may have up to thirty engraved slits, corresponding to the maximum number of photomultipliers available.

The spectrometer can be equipped with either a single or a dual spark stand of the argon-flushed or air type. One advantage of a dual spark stand, where the individual spark stand is pneumatically brought into the proper position with respect to the optical axis of the spectrometer, is that samples of different shapes may be used. Another advantage becomes evident in routine work: if the throughput of sample is very high, a single spark stand may be heated by the thermal energy released during sparking, and this could intro-

duce analytical errors. Changing from one stand to the other, which requires only 1 to 2 s, eliminates this source of error. Every sample is pneumatically clamped on the spark stand, so the spark gap width is very reproducible and rapid sample handling is possible.

The sensitivity of the photomultipliers can be adjusted very simply. Coarse step printed circuit boards make it possible either to select one of ten sensitivities by relocating a wire or to

choose new sensitivities for each of the five analytical programmes, thereby increasing the versatility of the system. By means of the computer, five fixed analytical programmes can be stored in the system. These, in turn, can be divided into any number of subprogrammes by means of punched paper tape. Thus, even in case of complex high-alloyed materials, examination becomes possible.

Manual operation of this system is

extremely simple. Introduction of the sample, selection of the programme and actuation of the starter button are the only operating steps which are carried out by the operator; calculations and printout of the results in per cent by weight are carried out automatically. Even for complex, high-alloy materials results are within the limit of conventional standard deviations—but are obtained within about 1 s.

WOLFGANG THOMICH

CORRESPONDENCE

Dingle's Answer

SIR,—While I accept and appreciate the apology on page 315 of this issue for the charge of dishonesty made in Professor Ziman's review¹ of my book, *Science at the Crossroads*, the circumstances are such that a further statement from me is called for so that the matter shall be rightly understood. It would, of course, be possible for me to have made false statements quite honestly, and readers of *Nature* might well infer, in the absence of comment from me, that such was the fact that had merely been expressed in the review by the wrong word. It is particularly necessary for me to remove that impression because the description of the book given by Professor Ziman, which has already been independently described in *Nature* as "admirable"², fails to make clear that its whole significance depends on the truth of its factual statements. "My purpose throughout", I wrote (page 19), "is not to indict but to inform, and let the facts bring whatever indictment is necessary." I cannot, therefore, justifiably allow any doubt to exist of the trustworthiness of those facts, for all of which I possess conclusive evidence.

This clarification is the more necessary because Ziman's opening sentence ("This is Professor Dingle's account of his attempt to persuade the scientific community that the theory of relativity is wrong and should be repudiated") must almost certainly suggest that to give such an account was the main, if not the only, purpose of the book. This would be quite false. I certainly should not have written it as a piece of autobiography, or even to try again to persuade the scientific community of anything. I wrote it, with great reluctance, only after 13 years of continuous effort in that direction had convinced me that further effort was useless, and it was because

the reason for my failure seemed to me so ominous, both for the future of science and for the effect of scientific research on public welfare generally, that I had a moral obligation to make the whole facts known, free from my interpretation of them, and to leave readers to form their own judgment of what they implied. I referred in the preface (page 9) to my inability to obtain "the one essential desideratum of the whole exercise—plain evidence, through an answer to, or acceptance of, a very simple refutation of the immeasurably important special relativity theory, that the obligation to preserve strict integrity in science continues to be honoured", and I began the summing-up at the end (page 219) by saying "The primary and inescapable purpose of this book, which Part One attempts to fulfil, is to make known, to those with an indefeasible right to the knowledge, the present state of the scientific world as revealed by its practice, and to bring it into comparison with what is generally believed, and implicitly trusted, to be its state as typically expressed by the late Sir Henry Dale. I leave the reader to judge the significance of the comparison for himself, and to estimate what the consequences are likely to be if the present degree of conformity continues." This purpose is continually stressed in the intervening pages.

To prevent further misunderstanding I must add that part 2 ("The Intellectual Issue") on which Professor Ziman does not comment, though of equal length to part 1 ("The Moral Issue"), is of relatively academic interest. It is my reading of the historical causes of the change of attitude of scientists to their work, and is, of course, open to free criticism which I have not the slightest desire to suppress. Part 1, being incontrovertibly factual, is open only to reflexion, leading to whatever action concerned readers may think it proper to take. Professor

Ziman's purported answer to my scientific "Question", which is the source and not the substance of part 1, has already been dealt with³.

Yours faithfully,

HERBERT DINGLE

Purley, Surrey

¹ Ziman, J., *Nature*, **241**, 143 (1973).

² Ellis, G. F. R., *Nature*, **242**, 143 (1973).

³ Dingle, H., *Nature*, **242**, 423 (1973).

Research Funding

SIR,—While it may appear fruitless to object to yet another cutback in federal funding for education and science, I do wish to protest very strongly about the recent reduction in a little noticed but extremely important programme—the National Science Foundation Undergraduate Research Participation programme.

Under grants from this programme, undergraduate science students conduct independent research projects during the summer months. The most gifted and dedicated students are exposed to the literature and techniques of the field, and are given the opportunity to design and execute their own projects under the aegis of an experienced professor.

This will be the fourth summer that I have supervised such a programme in the Biology Department at Massachusetts Institute of Technology. It is noteworthy that all of the students in this programme have gone on either to graduate school in the biological sciences or to medical school. Many of these students, while still undergraduates, published papers on their research or presented papers at national meetings.

My own interest in scientific research was kindled by four summers' research while in college and high school. My colleague Dr David Baltimore, a leader in cancer research and the study of

RNA tumour viruses, was first introduced to scientific research in NSF programmes. I could cite numerous similar examples from among the current leaders in virtually every field of scientific research.

This programme has been threatened several times with extinction. Funds for it were cut this year from \$3.9 million in 1972 (supporting 2,600 students) to \$2.0 million (supporting 1,300 students). The seriousness of this cut can be seen from the fact that, in all fields of the biological and medical sciences, and in all the colleges and universities in the nation, there will be space for only 308 students this year. Our own programme has been cut from 20 to 15 students. For these places we received over 160 applications and we were forced for lack of funds to reject many straight A and other very qualified students.

The scientific leaders of the USA during the next two decades are the gifted undergraduates of today; it is these students who are now being discouraged from entering research careers in all fields of science because of reductions of federal support in training and research funds at all levels. The NSF-URP programme, in particular, plays a central role in attracting gifted people into research careers, and its demise may have profound effects on the production of future scientific leaders.

Yours faithfully,

HARVEY F. LODISH

Department of Biology,
Massachusetts Institute of Technology,
77 Massachusetts Avenue,
Cambridge, Massachusetts 02139

Nobel Realpolitik

SIR,—No doubt Eugene Garfield (*Nature*, **242**, 485; 1973) is correct about the correlation of citation frequency and Nobel Prizes. Such is the *Realpolitik* of science. But this indicates little about the true scientific value of frequently cited articles, and almost nothing about the value of those less frequently cited.

The *SCI* is a splendid bibliographic tool and may be a sociological tool as well. But I trust that the "rationale for thousands of infrequently cited journals" will not in fact be questioned merely on Dr Garfield's evidence. He should recall that Mendel's great paper was buried in an obscure journal, and Sir William Bragg's first seminal works rattled around ineffectually for a time in then-unknown Australian periodicals. There are quite a few other examples.

Thanks to *SCI's* *Permuterm Subject Index* such oversights need not now happen, but equally thanks to many infrequently-cited journals more really very good (and some really very bad)

material gets into print. There is always the chance of another Mendel.

Yours faithfully,

JAMES FRIDAY

The Royal Institution,
21 Albemarle Street,
London W1X 4BS

Unanswered Questions

SIR,—One of your correspondents¹ asks those who, like myself, have referred to "flaws" and "unanswered questions" in evolutionary thought if they can supply examples. We certainly can—many of them. For space reasons I will quote only three.

Only four years ago a Darwinist grudgingly admitted that "Though it is nearly a century (*sic*) since Darwin wrote his treatise *On the Origin of Species*, there are still a few weak points in the theory of evolution. Often evolution seems to have made huge jumps, leaving no traces of any intervening steps and no hint that anything but the complete system could have functioned at all"².

It is surely incompatible with the scientific method to pretend that evolution is a proven fact, with these "huge jumps" unexplained. How did the whale evolve a complex nipple that enables it to suckle its young under water? This complex organ would have been worse than useless until it was fully developed, or Whale Junior would have received a mouthful of seawater instead of milk.

How did the giraffe evolve a long neck without first evolving the complex hydraulic control system that maintains an acceptable blood pressure in his brain whilst he lowers and raises his head? Or did he evolve his unique mechanism for regulating cerebral blood pressure before he evolved his long neck—and if so, how could such motiveless evolution have occurred?

Anadromous fish, grey whales and some species of migrating birds share an ability to find their way to an exact spot thousands of miles away. It is doubtful whether a submarine could equal their performance without the aid of shore-based radio beacons, even using today's highly sophisticated computer-based navigational systems. How did creatures so widely separated in the evolutionary tree as birds, fishes and mammals, all evolve these extraordinary navigational mechanisms? The problem is compounded by the experimental evidence that the spatial distribution of various elements in the oceans, the Earth's magnetic field, and the position of the stars all play a part in the overall phenomenon. Yet over geologic time these three parameters have been varying continuously; how, then, did these creatures evolve the ability to navigate against a moving datum?

If evolutionists would face these questions—and hundreds of others like them—honestly, they might both advance their branch of science and gain a little more respect for those Bible-believing scientists who see no incompatibility between science and their Christian faith.

Finally, let me point out that your correspondent is a couple of generations out of date in his glib assertion that "any archaeologist, theologian or philosopher could tell them that many of the stories in the Bible are copied from the folktales of long-ago tribes more ancient than the Hebrews".

As I have shown at some length³ there are two schools of thought even amongst theologians about the accuracy of the Old Testament's historical narratives—and a considerable proportion of the world's leading archaeologists now regard these narratives as highly accurate records that are independent of, and in many cases precede, the legends of other ancient peoples.

Yours faithfully,

A. T. J. HAYWARD

2 Wellington,
East Kilbride,
Glasgow G75 8RB

¹ Angseeing, J. P. A., *Nature*, **242**, 214 (1973).

² Anonymous, *New Scientist*, December 11, 542 (1969).

³ Hayward, A., *God's Truth: A Scientist Shows Why it Makes Sense to Believe the Bible* (Marshall Morgan and Scott, London, 1973).

Errata

THE title to the article by M. Koltai and E. Mees (*Nature*, **242**, 525; 1973) should read "Inhibition of the Acute Inflammatory Response by Interferon Inducers", not "... Interferon Inhibitors".

IN the article "Sexual Behaviour and Sexual Motivation in the Female Rat" by R. F. Drewett (*Nature*, **242**, 476; 1973) the first sentence in the last paragraph should read "Oestrus is associated with enhanced general activity but this seems to be independent of sexual receptivity".

IN the article "Sativin: an Induced Isoflavan from the Leaves of *Medicago sativa* L." by J. L. Ingham and R. L. Millar (*Nature*, **242**, 125; 1973) paragraph 11, line 14, should read "sativin" instead of "sativan" and paragraph 12, line 6, should read "lonchocarpan" instead of "lonchocarpin". The contents page entry should read "isoflavan" instead of "isoflavin" in both cases.

IN the article "Further Data on the Corsica-Sardinia Rotation" by F. Radiati di Brozolo and G. Giglia (*Nature*, **241**, 389; 1973) paragraph 23, line 11, "lower" should read "upper" and "upper" should read "lower".

Corrigendum

In the article "Cyclical Changes in the Habitat and Climate of an East African Ecosystem" by D. Western and C. van Praet (*Nature*, 241, 104; 1973) in Fig. 3, $r = -0.92$ and the equation should read, $\log y + 1 = 2.87 - \log x$.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

- Parliamentary and Scientific Committee. Annual Report for 1972. Pp. 28. (London: Parliamentary and Scientific Committee, 1 Buckingham Palace Road, 1973.) [132]
- The Zoological Record*, 1969, Vol. 106, Section 9: Mollusca. Compiled by the Staff of the Zoological Society of London. Pp. 190. £8; \$19.30. 1969, Vol. 106, Section 14: Protochordata, together with Pogonophora, Enteropneusta, Graptolithina, Pterobranchia and Phoronidea. Compiled by D. B. Carlisle and R. O. Connick. Pp. 49. £5; \$12. (London: The Zoological Society of London, 1972.) [132]
- Department of Trade and Industry. Domestic Meter Reading. By Atkins Planning. Pp. xi+118. (London: HMSO, 1973.) £2.35 net. [132]
- The Mathilda and Terence Kennedy Institute of Rheumatology. Fifth Annual Report 1971. Pp. 78. (London: The Mathilda and Terence Kennedy Institute of Rheumatology, Bute Gardens, W6, 1973.) [152]
- Strategy 2000: a Radical Approach to Pollution, Population and Resources. By R. S. Scorer. (First Series, No. 1.) Pp. 32. (London: Liberal Party, Liberal Bookshop, 7 Exchange Court, WC2, 1973.) 25p. [152]
- Estuarine and Coastal Marine Science*, Vol. 1, No. 1, January 1973. Edited by N. C. Fleming, B. H. Ketchum and E. Naylor. Pp. 1-111. Subscription: Volume 1 (4 issues), January-October 1973. Inland £10, postage 60p; abroad £11.50, postage 60p. (London and New York: Academic Press, 1973.) [152]
- Sixty-fifth Annual Report of the National Museum of Wales, 1971/1972. Pp. 147. (Cardiff: National Museum of Wales, 1972.) [162]
- The Year Book of the Royal Society of London 1973. (No. 77.) Pp. 448. (London: The Royal Society, 1973.) £2.10; \$5.90. [162]
- British Antarctic Survey. Scientific Reports No. 74: A Study of the Radio Aurora and Meteors at Halley Bay during the I.Q.S.Y. By Dr D. M. Shipstone. Pp. 68+1 plate. (London: British Antarctic Survey, 1972.) £2.50 net. [162]
- Business and a Behavioural Tradition. By Professor A. L. Minkes. (Inaugural Lecture delivered in the University of Birmingham on 21 November, 1972.) Pp. 20. (Birmingham: The University, 1973.) 25p. [192]
- Sixth Annual Report of the Institute of Development Studies at the University of Sussex, UK. Pp. 36. (Brighton: University of Sussex, 1973.) [192]
- Proceedings of the Royal Irish Academy. Vol. 73, Series A. No. 1: Distortion of Spiral-Like Mappings. By P. K. Kulshrestha. Pp. 1-6. 10p. No. 2: The Spectral Mapping Theorem for Quasicommuting Sys-

- tems. By R. E. Harte. Pp. 7-18. 24p. No. 3: On the Differential Equation for Spherical Functions on Certain Symmetric Spaces. Pp. 19-24. 12p. No. 4: The Gravitational Field on a Uniformly Rotating Sphere in Third Approximation. By J. D. McCrea. Pp. 25-45. 40p. Vol. 73, Section B. No. 1: Post-Glacial Vegetational History and Relative Land- and Sea-Level Changes in Lecale, Co. Down. By G. Singh and A. G. Smith. Pp. 1-52+plates 1-6. 93p. No. 2: Radiocarbon Dating of the Raised Beach at Woodgrange, Co. Down. By P. O. Dresser, A. G. Smith and G. W. Pearson. Pp. 53-56. No. 3: A Revised and Tabulated List of the Irish Hemiptera-Heteroptera—Part 1. Geocorisae. By N. MacNeill. Pp. 57-60. 6p. (Dublin: Royal Irish Academy, 1973.) [192]
- University of Oxford. Annual Reports 1970-1971. (Supplement No. 2 to the *University Gazette*, Vol. ciii, February 1973.) Pp. 19. (Oxford: The University, 1973.) 50p. [202]
- University of Cambridge. Report of the Sit-In, February 1972, and Its Consequences. (*Cambridge University Reporter*, Special No. 12.) Pp. 84. (Cambridge: The University, 1973.) 4p. [202]
- Inland Waterways Guide 1973 to Holiday Hire and Cruising Facilities. Edited by Robert Shopland and Charles Smith. Pp. 192. (Epsom: Boar World Publications; London: The Inland Waterways Association, 1973.) 38p. [212]
- Pollution in Four Industrialized Estuaries: Studies in Relation to Changes in Population and Industrial Development. (Four case studies undertaken for the Royal Commission on Environmental Pollution.) Pp. x+98. (London: HMSO, 1973.) 85p net. [212]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 273, No. 1237: The Statistical Properties of Echoes Diffracted from Rough Surfaces. By M. V. Berry. Pp. 611-658. £1.15; \$3.20. Vol. 274, No. 1238: Mount Etna and the 1971 Eruption. Edited by J. E. Guest and R. R. Skelhorn. Pp. 1-179+plates 1-3. £6.60; \$18.50. (London: The Royal Society, 1973.) [212]
- Loughborough University of Technology. Report to the University Court, 1971/1972. Pp. 121. (Loughborough: Loughborough University of Technology, 1973.) [222]
- Department of the Environment. Scottish Development Department. Welsh Office. Waste Disposal: Proposals for a New Framework. (Consultation Document.) Pp. 48. (London: Department of the Environment, 1973.) [222]
- PEP Broadsheet 540: Westminster to Brussels: The Significance for Parliament of Accession to the European Community. Pp. iii+28. (London: Political and Economic Planning, in association with the Hansard Society and the Study of Parliament Group, 1973.) 60p. [232]

Other Countries

- Studies on Free Energies of Mixing and Flow Activation Energy of Liquids. By M. M. Qureshi. Pp. vii+75. (Karachi: Pakistan Council of Scientific and Industrial Research, 1972.) [132]
- Sveriges Geologiska Undersökning. Stockholm. Lovö Geomagnetic Observatory Year Book 1971. Pp. 36. (Stockholm: Sveriges Geologiska Undersökning, 1972.) [132]
- Acide Abscisique: Structure, Synthèses et Métabolisme. Par C. Bulard, D. Fries, Th. Gaspar et R. Verbeek. Pp. 42. (Bruxelles: Académie Royale de Belgique, 1972.) [132]
- Texas Instruments, Inc. 1972 Annual Report. Pp. 8. (Dallas: Texas Instruments, Inc., 1973.) [142]
- Southwest Research Institute. Technical Activities Summary. Pp. 54. (San Antonio, Texas: Southwest Research Institute, 1973.) [142]
- The African Journal of Tropical Hydrobiology and Fisheries*, Vol. 1, No. 1, 1971. Pp. 1-83. Published bi-annually. Subscription rates: within East Africa, Sh.35; outside East Africa, East African Sh.70; US \$10. (Nairobi: East African Literature Bureau, PO Box 30022, 1971.) [142]
- World Data Center A for Solar-Terrestrial Physics. Report UAG-23: URSI Handbook of Ionogram Interpretation and Reduction. Second edition. Edited by W. R. Piggott and K. Rawer. Pp. vi+324. (Asheville, North Carolina: National Climatic Center, Federal Bldg., 1972.) \$1.75. [142]
- Canada: Ministry of State for Science and Technology. Annual Report 1971/1972. Pp. 11. (Ottawa: Ministry of State for Science and Technology, 1973.) [152]
- Calouste Gulbenkian Foundation. Preview 1973: Policies and Activities; Projects Initiated during 1971 and 1972. Pp. 58. (Lisbon: Calouste Gulbenkian Foundation, United Kingdom and British Commonwealth Branch, 1973.) [162]
- Comité International des Poids et Mesures. Comité Consultatif de Thermométrie, 9e Session—1971 (6-7 Juillet). Pp. 151. (Sevres, France: Bureau International des Poids et Mesures, 1973.) [202]
- Berichte des Deutschen Wetterdienstes, Nr. 129 (Band 17): Das Globale Luftdruckfeld in Seehöhe. Von Lothar Kaufeld. Pp. 57. (Offenbach a.M.: Selbstverlag des Deutschen Wetterdienstes, 1972.) [202]
- Smithsonian Contributions to Zoology. No. 135: Freshwater Triclad (Turbellaria) of North America. V: The Genus *Polycelis*. By Roman Kenk. Pp. iii+15. 45 cents. No. 137: A Taxonomic Hierarchy and Checklist of the Genera and Higher Taxa of Marine Nematodes. By W. D. Hope and D. G. Murphy. Pp. iii+101. \$2. (Washington, DC: Smithsonian Institution Press, 1972 and 1973. For sale by US Government Printing Office.) [202]
- US Department of the Interior: Geological Survey. Water-Supply Paper 1938: Water Supply for the Nuclear Rocket Development Station at the US Atomic Energy Commission's Nevada Test Site. By R. A. Young. Pp. iii+19. Professional Paper 689: Sedimentary Processes and Distribution of Particulate Gold in the Northern Bering Sea. By C. Hans Nelson and D. M. Hopkins. Pp. iii+27+plate 1. 75 cents. Professional Paper 749: Distribution of the Middle Ordovician Copenhagen Formation and Its Trilobites in Nevada. By Reuben James Ross, Jr., and Frederick C. Shaw. Pp. iii+33+plates 1-8. 70 cents. (Washington, DC: Government Printing Office, 1972.) [202]
- Report of the South African Museum for the year ended 31st March 1970. Pp. 17. South African Museum. Report of the Trustees for the year ended 31st March 1971. Pp. 16. (Cape Town: South African Museum, 1972 and 1973.) [212]
- Patterns of Prescription Drug Use: The Role of Promotion. By C. Joseph Stetler. Pp. 23. (Washington, DC: Pharmaceutical Manufacturers Association, 1155 Fifteenth Street, NW, 1973.) *Gratis*. [222]
- Science Council of Canada. Report No. 19: Natural Resource Policy Issues in Canada. Pp. 59. (Ottawa: Information Canada, 1973.) \$1.25. [222]
- Geological Survey of Canada. Paper 71-31: An Evaluation of Surface Geochemical Prospecting for Petroleum, Olds-Caroline Area, Alberta. By R. G. McCrossan, N. J. Ball and L. R. Snowdon. Pp. vii+101. \$3. Paper 71-49: Geology and Engineering Description of the Soils in the Welland-Port Colborne Area, Ontario. By E. B. Owen. Pp. iii+7. \$1.50. (Ottawa: Information Canada, 1972.) [222]

HOW TO BUY NATURE

The cost of one year's subscription to NATURE is:

	UK & elsewhere	US & Canada
Nature (Friday)	£16	£20
Nature & Nature Physical Science or Nature New Biology	£24	£33
Nature New Biology or Nature Physical Science	£10	£15
All three editions	£29.50	£44
Index (1972)	£1	£1.50

(Charges for delivery by air mail on application). Subscribers in North America may be able to claim a tax rebate against their NATURE subscription.

Editorial, Advertising and Publishing Offices of NATURE

MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2R 3LF
Telex 262024

MACMILLAN JOURNALS LIMITED
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number: 202-737 2355. Telex 64280

International Advertisement Manager

PETER R. KAVANAGH
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Numbers: UK 01-836 6633
USA 202-737 2355

Subscription Department

MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS RG21 2XS
Telephone Number: Basingstoke 29242

Classified advertisements

T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone Number: 01-242 6264/01-405 4743
Telegrams: Textualist London WC2A 2ED
Registered as a newspaper at the Post Office